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DEMONSTRATION OF ACCEPTABLE SYSTEMS FOR LAND DISPOSAL OF SEWAGE SLUDGE

By

Ohio Farm Bureau Development Corporation
Columbus, Ohio 43216

and

Ohio State University
Columbus, Ohio 43210

Cooperative Agreement No. CS 805189

Project Officer

G. K. Dotson
Wastewater Research Division
Water Engineering Research Laboratory
Cincinnati, Ohio 45268

This study was conducted in cooperation with the
Toxicology and Microbiology Division,
Health Effects Research Laboratory,
Cincinnati, Ohio 45268

WATER ENGINEERING RESEARCH LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
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Columbus, Ohio 43216

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The objective was to demonstrate sludge application systems for farmland that would minimize any adverse effects on the environment and public health, achieve both urban and rural acceptance, and be generally beneficial for producer and receptor of the sludge. A comprehensive health effects study of the families living on sludge-receiving farms was conducted. Health status of residents of 47 sludge-using farms were compared with 46 control farms. Neither incidence of disease, nor evidence of viral infections differed significantly between sludge-using and control farms. Neither was the health of livestock found to be different between the two groups of farms. The sludge was effective in increasing crop yields over yields without sludge or fertilizer.

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FOREWORD

The U.S. Environmental Protection Agency is charged by Congress with protecting the Nation's land, air, and water systems. Under a mandate of national environmental laws, the agency strives to formulate and implement actions leading to a compatible balance between human activities and the ability of natural systems to support and nurture life. The Clean Water Act, the Safe Drinking Water Act, and the Toxics Substances Control Act are three of the major congressional laws that provide the framework for restoring and maintaining the integrity of our Nation's water, for preserving and enhancing the water we drink, and for protecting the environment from toxic substances. These laws direct the EPA to perform research to define our environmental problems, measure the impacts, and search for solutions.

The Water Engineering Research Laboratory is that component of EPA's Research and Development program concerned with preventing, treating, and managing municipal and industrial wastewater discharges; establishing practices to control and remove contaminants from drinking water and to prevent its deterioration during storage and distribution; and assessing the nature and controllability of releases of toxic substances to the air, water, and land from manufacturing processes and subsequent product uses. This publication is one of the products of that research and provides a vital communication link between the researcher and the user community.

Potential soil contaminants such as wastewater sludge may become beneficial soil amendments when they are properly treated and applied to land. This project demonstrates systems for managing sewage sludge application to farm lands and investigates sludge-related health risks to rural residents and their livestock. The study addresses the concerns of the rural community and demonstrates that large municipalities can work cooperatively with large numbers of farmers in a mutually beneficial program.

Francis T. Mayo
Director
Water Engineering Research Laboratory

PREFACE

Sewage Sludge -- a valuable resource that has been mishandled, misunderstood and mismanaged. The application of sludge on farmland has been practiced for years in the U.S. Growing concern from farmers and the public about the sociological, environmental, soil and health risks associated with land application caused the Ohio Farm Bureau Development Corporation to initiate research studies that would define the risks and recommend management solutions where practical.

Portions of this study were subcontracted to the Ohio State University Research Foundation and the Ohio Agricultural Research and Development Center.

Jack K. Hill
Vice President of Operations
Ohio Farm Bureau Development Corporation

ABSTRACT

This project demonstrates management systems for land application of sewage sludge that minimize adverse impacts on the rural community in Ohio. Also, sludge related health risks to rural residents and their livestock are investigated.

The study demonstrated that large municipalities can work with large numbers of farmers on a cooperative basis in a mutually beneficial program. A model contract was developed for use by farmers and sludge generators. The sludge-receiving farms were distributed over a large area so that no rural residents would be forced to live in the vicinity of a permanent disposal site. Annual application rates ranged from 4 to 10 dry metric tons per hectare to permit efficient use of the plant nutrients without danger of runoff or ground water pollution. Applications were made throughout the year on privately owned lands used in the production of corn, soybeans, hay and wheat. The project was successful in all areas except Pickaway County, where the board of health issued an injunction against sludge application.

In support of the management effort, studies were conducted regarding soil compaction, nitrogen mineralization and volatilization, PCB-amended sludges, economics of sludge applications, and sludge quality.

The general health of residents from 47 sludge-receiving farms were compared with residents of 46 control farms. Questionnaires were completed on human and livestock health. Blood and fecal samples were collected for microbiological testing. Tuberculin testing was completed. Intensive observations were made of beef cattle herds on sludge and control farms.

Health risks were not significant when sludge was applied at the low application rates of this study using the management systems described here.

The risks of respiratory illness, digestive illness, infection with *Salmonella*, *Shigella* sp., and *Campylobacter* sp., and general symptoms of illness were not significantly different between sludge and control groups. Similarly, no significant differences occurred in the health of domestic animals on sludge and control farms. Fecal Cd levels in humans were not significantly affected by sludge.

Significantly higher fecal Cd concentrations were detected in cattle, and significantly higher Cd and Pb accumulations were observed in kidney tissues of calves grazing on sludge-amended pastures. The potential use of dietary zinc to reduce the cadmium body burden of food animals was demonstrated.

Procedures were developed for viral isolation from sludge. Serum neutralization tests were completed for viral infections. Viral infections among household members were observed. There was no significant difference in frequency of viral infections between sludge and control groups.

This report was submitted in fulfillment of Cooperative Agreement No. CS 805189 by the Ohio Farm Bureau Development Corporatioon under the sponsorship of the U.S. Environmental Protection Agency. This report covers the period October 1977 to June 1983, and work was completed as of April 1985.

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INTRODUCTION

The application of sewage sludge to farm lands is commonly practiced throughout the United States. The Water Pollution Control Act Amendments of 1972 and the Marine Protection, Research and Sanctuaries Act of 1972 has placed considerable emphasis upon this method of sludge disposal. The potential to convert this waste material into a useful fertilizer has appeal to all who are interested in conservation of our natural resources.

The management of the application of sewage sludge on farm lands in Ohio has most generally been conducted in a satisfactory manner. However, over the years there have been incidents which have had negative impacts upon the rural community. In the name of emergencies at the sewage plant very odorous sludges have been applied to land in an unsatisfactory manner. At least one Ohio farmer had unknowingly received sludge which contained excessive levels of heavy metals. Some sludge haulers still do not appreciate the need for care that sludge is not spilled from trucks along the highway. Some sludge generators would still prefer to acquire land through eminent domain for the establishment of a dedicated sludge disposal farm. Fortunately these are rare exceptions in Ohio's experience regarding land application of sludge.

The concern over these potential problems and health risks associated with the application of sludge to land are still real issues for many Ohio farmers. As recipients of the sludge on their land or in their neighborhoods Ohio farmers are deeply involved. The Ohio Farm Bureau Federation which is a membership organization representing 98,000 Ohio farm families has been involved in the resolution of some conflicts between sludge generators and farmers. The membership of the Ohio Farm Bureau Federation have also repeatedly voted to sponsor or seek support for a project which would demonstrate management systems which give due consideration to the concerns of the rural community and which would more clearly define the health risks to the rural community and their livestock.

The Ohio Farm Bureau Federation would like to express its appreciation to the United States Environmental Protection Agency for its support of this project which is so important to Ohio farmers and farmers throughout the United States.

CONCLUSIONS

A major objective was to define and demonstrate management systems for application of sludge to farm lands, which would minimize the adverse impacts on the rural community. The key factors in management of land application of sewage sludge on privately owned farm land are as follows:

A. The involvement of a large number of farmers and application sites so that the general public will not identify a particular farm or neighborhood as the sludge disposal site.

B. Public meetings, consultation with community leaders, field days, etc., to make the public fully aware of the scope, objectives, and safety of the program. The residents of Ohio were generally supportive of the concept of applying sludge to farm land as long as there was a considered management approach which minimized odor problems, avoided nuisance situations in the transport and handling of the sludge, and maintained the metal content of the sludge at reasonable levels.

C. Low application rates which provide sufficient sludge for either the nitrogen or phosphorus requirements of crops. This concept is readily accepted by the public. It provides for efficient utilization of the plant nutrients of the sludge. It also minimizes the potential for surface runoff and ground water pollution since the level of nutrients applied are comparable to fertilizer applications on non-sludge treated land. It minimizes the possibility of damages resulting from the application of unwanted metals or organics to land resulting from the unlikely failure of the program for monitoring the quality of the sludge.

D. The development of a rapport between the people involved in spreading the sludge and the farmers who receive the sludge. A management program requires someone versed in agronomy to serve as a liaison between farmers and the sludge generator. This individual would discuss with the farmer the nutrient value of the specific loads of sludge to be received. He would also present and discuss monitoring data on the heavy metal content of the sludge. He would present a contract to farmers which would define the working relationship between the farmer and sludge generator. In general he would try to trouble shoot and maintain a good relationship between farmers and sludge generators.

E. Careful monitoring of the quality of the sludge and care to produce a well stabilized, odor free sludge. Odorous sludges do arise when sewage plants are not functioning properly. The disposal of such sludges on the land must not be considered to be a necessary emergency procedure, which the public must simply accept. A plan for such situations should be worked out ahead of time. At a minimum odorous sludges should be incorporated into the soil as they are applied to the land.

As long as these practices were followed carefully we were able to maintain a good relationship between farmers and cities of a few thousand people to major metropolitan areas such as Columbus, Ohio. Large volumes of sludge were applied to the land at low application rates with very few complaints about the operation from the public.

Problems were encountered when the City of Columbus applied a very odorous sludge to farms in Pickaway County. This was considered to be an emergency effort by Columbus officials and was not conducted as a portion of our project. Unfortunately there was a breakdown in communications between the project staff and the city regarding this application. The end result was an injunction by the Pickaway County Board of Health against all land application of Columbus sludge within Pickaway County. The only conclusion to be drawn from this experience is that application of odorous sludges to the land requires very careful planning. The only acceptable approach is to incorporate the sludge as it is applied to the land.

The second major objective was to evaluate health risks to rural residents and their livestock resulting from the application of sludge to crop land. We all encounter exposure to germs and viruses as we associate with other people during our daily lives. The objective of this study was to determine if the presence of sludge on land in the rural community would increase the risk of disease above the risks associated with our daily living. Literature reviews of the risk of disease associated with sewage sludges are presented in sections 11 and 15.

It was concluded that health risks were not significant when sludge is applied at the low application rates of this study and using the management systems of this study. The risks of respiratory illness, digestive illness, Salmonellae, exposure to Shigellae sp. and Campylobacter sp. or general symptoms were not significantly different between sludge and control groups. Similarly there were no significant differences in the health of domestic animals on sludge and control farms. Viral infections among household members were observed. There was no significant difference in frequency of viral infections between sludge and control groups. Fecal Cd levels in humans were not significantly affected by the exposure of rural residents to sewage sludges.

Agronomic studies were conducted to support and evaluate the land application program. Field plots were maintained to demonstrate that crop yield response to sludge, metal accumulation in soils and metal accumulation in plant tissue under Ohio conditions were typical of results reported in the literature. This information was used in educational programs for the public and also for sanitary engineers, public health officials and other interested professionals.

The metal content of six sludges were monitored over a period of three to four years. Sludge samples were collected on a daily basis and analysis was performed on a monthly composite. These analyses were considered sufficient for monitoring the quantities of metals which might be applied to soils. Compositions were reasonably steady throughout this time period. Abrupt changes were only observed when sewage plant modifications were started up or when industrial pretreatment programs were initiated.

Soil compaction studies were conducted in the Medina project to evaluate the possible damage to soil structure resulting from the travel of application vehicles through fields. This issue requires further study since it was only feasible for us to observe compaction effects resulting from one type of application vehicle on one soil type. Also the application rates in the Medina area were held fairly low, requiring only one or two passes of the application vehicle to provide the total annual application. As an overall conclusion it appeared that soil compaction due to the sludge application equipment was not of great concern under the conditions of the Medina project.

Laboratory studies of nitrogen mineralization and ammonia volatilization losses from soils amended with sewage were conducted with a number of Ohio sludges. It was concluded that nitrogen mineralization of sludge in soils was extremely variable depending upon the sludge and field conditions. The mineralization rate of 30 percent when sludge was first applied to the land was used in our agronomic recommendations.

A number of parameters which influence the volatilization of ammonia from sludge amended soils was investigated. The most important observation relating to our management approach was that ammonia is quickly lost when sludges are applied to soil surfaces. In this project sludges were applied to the surface of soils and often remained on the surface for a week to a couple of months prior to incorporation into the soil. It was generally assumed that most of the ammonia nitrogen was lost. Such losses cannot be avoided when applications are made to hay and pasture lands and on fields which support application equipment but are not in a suitable condition for tillage at the time of the application.

A laboratory investigation of the factors affecting soil degradation and plant absorption of PCB from PCB amended sewage sludge was conducted. The sludges used in this demonstration were not contaminated with PCB. Yet there is always concern regarding the possibility that PCB would reach the land as a result of the application of sewage sludge. It was observed that PCB was resistant to biodegradation in soils. Volatilization from soils was decreased by the organic component of soils. Uptake of PCB by Kentucky 31 fescue was very limited. The possibility of PCB and other toxic organics reaching crop land is an issue of concern to farmers who receive sludge. More research is needed regarding the hazards associated with the presence of these materials in the soil. Less expensive and more reliable methods for monitoring the presence of toxic organics in sludge is also needed.

A survey of the state of the art of land application of sludge in Ohio was completed. A total of 56 landspreading communities were identified in Ohio. The quality of land spreading programs in Ohio has been improved substantially over the past five years. Communities are more aware of the contents of their sludges and spread it in a more judicious manner.

An economic analysis of land spreading of sludge was completed. The analysis was prepared in a computer format so that the specific conditions of a given community could be quickly evaluated. Landspreading of sludge is an economic method of disposal for most Ohio communities.

The effect of soil pH on the extractability of Cd was observed for several Ohio soils and sludges in laboratory studies. The movement of Cd from sludge treated soils into the food chain is a concern. The extractability of Cd in sludge amended soils increased dramatically as the pH of the system dropped below 6.0.

SECTION 1

GENERAL DESCRIPTION OF THE STUDY AREAS

Terry J. Logan, B.S., M.S., Ph.D.
C. Richard Dorn, D.V.M., M.P.H.
Chada S. Reddy, B.V.Sc., M.S., Ph.D.
The Ohio State University
Columbus, Ohio 43210

SELECTION OF STUDY SITES AND TREATMENT PLANT CHARACTERISTICS

When this project was first envisioned, the objective was to demonstrate and study optimum land application methods with systems that varied with regard to: type of sewage treatment, size of community, soils and cropping practices. These factors were used to screen potential candidates and a number were further investigated as to their willingness to participate. The original sites selected were City of Columbus, Medina County, City of Defiance and City of Zanesville. Zanesville was dropped from the project after one year because its sludge contained cadmium at concentrations between 200-400 µg/g, too high to be considered for a successful land application program, and because the specific source or sources of the metal could not be identified. The City of Springfield was then chosen as an alternative. The locations are given in Figure 1.1.

Table 1.1 shows that the four study sites vary considerably in population served and sludge produced. They also offer some differences in type of sewage treatment, and they differ considerably in their nutrient and metal contents (Table 1.2). Columbus uses anaerobic digestion followed by centrifugation to 17-22 percent solids. Springfield and Defiance both have anaerobic digestion and both pump directly from the digestor to the sludge truck. Springfield also pumps liquid digested sludge to lagoons and some of the lagooned sludge has been land applied. Medina has a county-wide system which employed three small aerobic digestion plants (100, 300, 500) during the project. The City of Medina was not served by either of these plants during the study, but was brought on line in September 1980. The impact this had on metal concentrations in the sludge is discussed later in the report.

The four study sites also varied considerably in type of transport and sludgespreading equipment, and degree of previous experience with land application. Prior to the initiation of this project in 1978, Columbus has had no systematic land application program--most of the sludge has been lagooned or landfilled. In designing the land

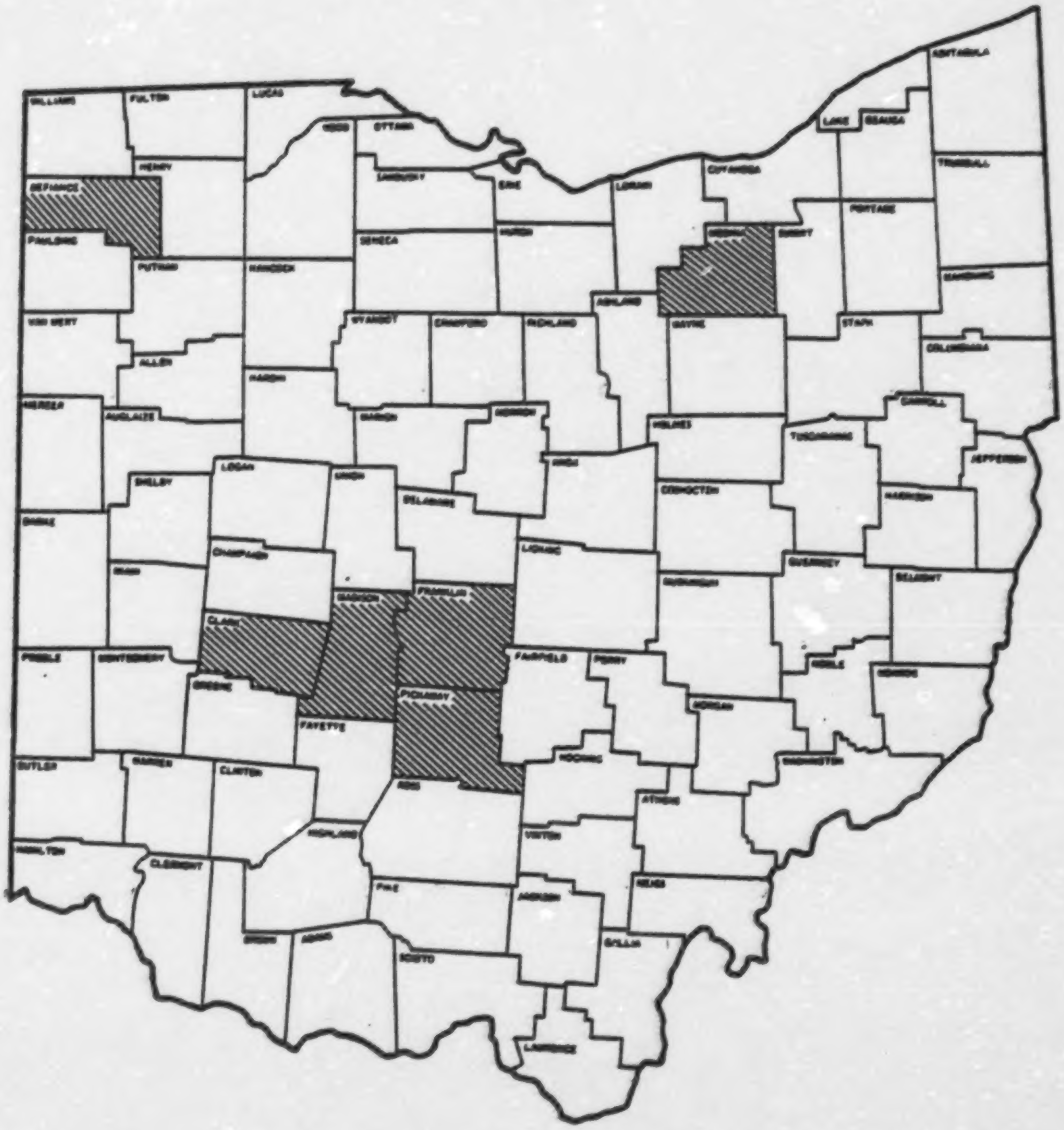

WILLIAMS
FULTON
LUCAS
WOOD
OTTAWA
HENRY
DEFIANCE
SANDUSKY
ERIE
LORAIN
CUYAHOGA
LAKE
GEAUGA
ASHTABULA
TRUMBULL
PAULDING
PUTNAM
HANCOCK
SENECA
HURON
MEDINA
SUMMIT
PORTAGE
MAHONING
VAN WERT
ALLEN
WYANDOT
CRAWFORD
RICHLAND
ASHLAND
WAYNE
STARK
COLUMBIANA
MERCER
AUGLAIZE
HARDIN
MARION
MORROW
HOLMES
TUSCARAWAS
CARROLL
JEFFERSON
SHELBY
LOGAN
UNION
DELAWARE
KNOX
COSHOCTON
HARRISON
DARKE
CHAMPAIGN
MIAMI
LICKING
GUERNSEY
BELMONT
MUSKINGUM
MADISON
FRANKLIN
CLARK
PREBLE
MONTGOMERY
GREENE
FAIRFIELD
PERRY
NOBLE
MONROE
PICKAWAY
FAYETTE
MORGAN
BUTLER
WARREN
CLINTON
HOCKING
WASHINGTON
ROSS
ATHENS
HIGHLAND
VINTON
HAMILTON
CLERMONT
PIKE
JACKSON
MEIGS
BROWN
ADAMS
SCIOTO
GALLIA
LAWRENCE

Figure 1.1. Counties (hatched lines) in Ohio which were involved with the land application project.

TABLE 1.1. CHARACTERISTICS OF THE MUNICIPALITIES AND SEWAGE TREATMENT PLANTS IN THE STUDY

Municipality	Treatment Plant Name	Approximate Population Served	Sludge Produced (dry metric tons/year)	Type of Treatment	Sludge Transport and Land Application
City of Columbus	Jackson Pike	600,000	22,600 (8000)	Anaerobic digestion Centrifuge dewatering	Tractor trailer hopper truck for hauling; Ag Chem Terragator five wheel spreader. Flotation tires. Box spreader.
City of Defiance	Defiance	17,000	900	Anaerobic digestion	Farm tractor pulled liquid manure spreader. Also 8000 liter tank truck spreader with flotation tires. No transport vehicles used.
Medina County	Medina, 100, 300, 500	48,000	600	Aerobic digestion	Nurse tank truck for hauling. Eleven thousand liter tank truck spreader with flotation tires.
City of Springfield	Springfield	80,000	1,800	Anaerobic digestion	Dual wheel tank trucks (8000 liter) for hauling and spreading. No flotation tires.

TABLE 1.2. CHARACTERISTICS OF THE SLUDGES USED IN THE STUDIES (AVERAGE FOR 1978-1982)

Treatment Plant	Solids	pH	NH_3-N	TKN	P	K	Cu	Cd	Pb	Ni	Zn	Cr
	%		mg/kg									
Jackson Pike	3.8(18.1)*	7.7	11,838	40,066	24,121	3,819	733	77.8	557	375	5223	109
Defiance	3.3	7.4	20,690	51,020	26,990	5,908	340	7.6	386	143	1058	102
Medina 100	1.6	6.9	4,198	41,483	24,934	10,400	709	9.4	257	34	970	151
Medina 300	3.5	6.8	1,469	29,429	41,920	5,803	461	4.7	111	26	754	175
Medina 500	2.2	6.9	2,244	33,020	29,548	9,677	345	17.3	133	30	797	153
Springfield	7.3	7.4	9,748	31,308	18,550	4,697	746	42.4	615	544	8899	75

* Solids content of the dewatered cake sludge.

application program, Columbus decided to dewater sludge to reduce transportation costs. Most of the land application sites are located in southern Franklin and northern Pickaway Counties within 25 km of the treatment plant. Because of the special needs of the health study, some sites were located further away than would normally be considered. The sludge was transported to the field in open-top hopper tractor trailers and the sludge was stockpiled at the spreading site. A front-end loader was used to load the cake sludge into the spreader, a five-wheel Ag-Chem Terragator with flotation tires and a hopper box. Landspreading in Columbus was done by a contractor, S and L Fertilizer Company, and this operation is discussed in more detail elsewhere.

The Defiance program was on-going when they became part of the project. Liquid sludge is spread on city-owned land at the treatment plant with a farm tractor and a liquid manure wagon. Corn and hay are grown in rotation. Since their equipment was not suitable for over-the-road operations, they purchased an 8000 liter liquid sludge truck with flotation tires. Defiance was not part of the health study, and only three private farms were used during the project.

Medina County had just purchased a liquid sludge truck with high flotation tires and was just starting its land application program when it joined the project in 1978. It uses liquid sludge tank trucks to haul to the landspreading site.

The City of Springfield had been land applying part of its liquid digested sludge for at least 10 years prior to joining the project. They use dual wheel trucks for hauling and spreading. As discussed later, the lack of flotation tire equipment has hampered their landspreading activities in wet weather.

AGRICULTURAL CHARACTERISTICS OF THE STUDY AREA

Columbus

The Jackson Pike sludge was spread in southern Franklin, and northern Pickaway Counties (Figure 1.1). The treatment plant is located in southern Franklin County, and although the city is rapidly growing into this area, there is still considerable acreage of farmland available for landspreading. However, there are more restrictions on potential landspreading sites in Franklin County than in Pickaway County because of the greater density of homes in Franklin County. Landspreading of Columbus sludge was terminated in Pickaway County by the county health department because of bad publicity generated by the emergency application of raw primary sludge from Columbus' other treatment plant on several farms in Pickaway County in 1980.

The agriculture in Franklin and Pickaway Counties is general grain farming with corn, soybeans and winter wheat the dominant crops. There is also some hay and pasture land for dairy and beef operations. There is also a small amount of hog production and, in southern Franklin County, some nursery crop production. The soils are derived primarily from glacial till and outwash deposits of recent origin (<18,000 years) and are primarily Alfisols and Mollisols. Their major limitation to land application of sewage sludge is seasonal wetness.

Defiance

The sewage treatment plant is located in the southeastern part of the County on the eastern edge of the city (Figure 1.1). There is land available for spreading within 2-4 km of the treatment plant. Agriculture in the area is almost exclusively grain farming with soybeans, corn and winter wheat the major crops. There is very little livestock in the area. The soils are derived from recent glacial till and glacial lacustrine sediments. They are Inceptisols and Mollisols with clay to clay loam textures. Very poor drainage is the major limitation to landspreading.

Medina

This program involved landspreading sludge from three plants on sites throughout the County (Figure 1.1). The urban population density is not high, with City of Medina the only major town. However, there are smaller towns and villages and suburban strip housing throughout the County. The agriculture in the area is a mix of grain and dairy farming with some beef cattle. The major crops are corn and hay and/or pasture. There are some small grains grown, primarily winter wheat and spring oats, and there is an increasing acreage of soybeans. The soils in the area are derived from low lime glacial till and are mostly Alfisols, Mollisols and Inceptisols. Slope and erosion and, to a lesser extent, drainage are the factors most limiting sludge application.

Springfield

Sludge from the city of Springfield has been spread on farms throughout Clark County (Figure 1.1). There is considerable agricultural land within 10-15 km of the city limits that is suitable for landspreading. The agriculture in the County is general grain farming with corn, soybeans and wheat the major crops. There is some dairy and hog farming, and a few small beef-cattle operations, and a limited acreage of hay and pasture crops. The soils are derived from high lime glacial till and are Alfisols and Mollisols. Poor drainage is the factor most limiting to land application of sewage sludge.

GENERAL ASPECTS OF THE HEALTH STUDY

The general objective of the health study was to determine the risk to farm families of exposure to low rates of digested sewage sludge applied to their land. The overall approach was to monitor the presence of bacterial, viral and parasitic pathogens in sludge and in stool and blood specimens of members of the farm families receiving sludge. A control group of farm families was similarly tested. Candidate families were selected in each study area from a pool of local families who had indicated a willingness to participate. The sludge-receiving and control families were assigned at random from the pool. Table 1.3 gives the number of farms and participants in the sludge-receiving and control groups for each area, and the acreage and rate of sludge application for each farm is given in Tables 1.4-1.6. Defiance was not included in the health study, and the largest number of participants were in the Columbus group. The effects of sludge exposure on livestock health were also studied at a limited number of farms in the three study areas. Details of the health studies will be published elsewhere.

AGRICULTURAL DEMONSTRATION PLOTS

In each of the four study areas, demonstration plots were established on farmers' land to compare sludge to commercial fertilizer. In some cases, sludge alone was compared with fertilizer, but in most cases, sludge was used as a supplement to fertilizer. In addition to the farm plots, a five-year replicated study with Jackson Pike sludge was conducted at the Ohio State University Farm Science Review in Columbus to compare the effects of two rates of sludge versus fertilizer and a control on yield and metal uptake by corn, soybeans and wheat.

There were a total of 27 demonstrations in the four areas involving 18 farms and six crops (corn, soybean, wheat, oats, alfalfa hay and legume/grass hay). The average rate of application varied from 2-10 metric tons/hectare (Table 1.3) and the average size of fields which received sludge was 47 hectares for Columbus, 15 hectares for Medina and Springfield and 5 hectares for Defiance. Rates of sludge application and nutrients and metals added in sludge were monitored as was yield and metal content of diagnostic leaves. In addition, total and DTPA-extractable metals were determined on a random sample of all sludge-receiving farms and from all demonstration plots before sludge application and at the termination of the study.

SPECIAL STUDIES

A number of special studies were conducted in support of the general objectives of this project. They are reported and follow as individual sections and appendices.

TABLE 1.3. CHARACTERISTICS OF THE LANDSPREADING STUDIES FOR THE ENTIRE PROJECT PERIOD (1978-82)

Location	Control		Sludge Receiving		Total Hectares Receiving Sludge	Total Dry Metric Tons Sludge Applied	Average Rate (dry metric tons/ha)
	Farms	Participants	Farms	Participants			
City of Columbus (Franklin, Pickaway, Madison County)	25	76	25	101	1,176	11,677	9.9
City of Defiance* (Defiance County)	--	--	3	--	15	93	6.2
Medina County	10	26	11	31	167	333	2.0
City of Springfield (Clark County)	13	35	11	32	167	753	4.5

* Not involved in the health study. Most of the sludge is spread on city-owned land at the treatment plant.

TABLE 1.4. RATES AND ACRES OF SLUDGE APPLICATION ON FRANKLIN AND PICKAWAY COUNTY FARMS*

Farm Code	Date of Application	Dry Metric Tons Applied	Hectares	(Dry Metric Tons/Hectare)
3002	6/20/79	6.1	1.4	4.4
	10/27/80	18.0	2.8	6.4
	1/13/81	33.6	2.8	12.0
3003	11/5/79	118.0	14.5	8.1
	9/8/80	123.0	9.7	12.7
	10/12/81	161.3	12.1	13.3
3004	7/23/79	14.1	3.0	4.7
	8/7/80	242.5	24.2	10.0
	1/21/81	465.7	50.1	9.3
	11/13/81	683.0	46.9	14.6
3005	7/26/79	34.1	8.5	4.0
	2/6/80	21.6	2.0	10.8
	6/23/80	81.9	10.5	7.8
	8/20/81	14.0	3.2	4.4
	10/20/81	121.9	9.7	12.6
	2/10/82	98.6	8.5	11.6
3006	8/1/80	1228.6	127.3	9.7
	7/13/81	826.9	53.3	15.5
	1/13/82	680.9	45.7	14.9
3007	10/1/80	211.5	27.5	7.7
	3/26/81	1209.2	108.3	11.2
	10/14/81	575.3	58.6	9.8

TABLE 1.4. CONTINUED

Farm Code	Date of Application	Dry Metric Tons Applied	Hectares	(Dry Metric Tons/Hectare)
3009	3/12/79	21.8	4.0	5.5
	9/30/80	57.9	4.8	12.1
	1/11/82	52.3	4.8	10.9
4001	6/17/80	55.7	6.5	8.6
4002	11/20/79	162.4	27.1	10.0
	8/28/80	596.0	52.5	11.4
	8/31/81	265.6	24.2	11.0
4003	1/29/80	147.5	12.9	11.4
4004	10/9/78	45.5	7.0	6.5
	3/27/79	111.7	6.5	17.2
	6/27/79	34.6	7.9	4.4
	4/22/80	170.0	17.0	10.0
4006	2/20/80	388.0	36.8	10.5
4007	11/28/79	142.7	14.1	10.1
4008	2/29/80	158.1	18.2	8.7
4009	12/17/79	89.2	9.7	9.0
	7/29/80	165.0	16.2	10.1

TABLE 1.4. CONTINUED

Farm Code	Date of Application	Dry Metric Tons Applied	Hectares	(Dry Metric Tons/Hectare)
4010	2/6/80	157.1	15.4	10.3
4011	2/11/80	74.2	18.2	4.0
4012	10/9/79	376.8	64.2	5.8
	5/12/80	398.8	24.6	16.1
4013	11/18/79	89.2	14.1	6.3
	5/12/80	3.9	0.4	9.6
4014	2/14/80	55.6	10.1	5.4
4016	2/25/80	58.5	6.9	8.5
	6/24/80	333.9	52.9	6.3
4017	10/18/78	66.5	10.2	6.5
	5/30/79	42.4	9.5	4.5
	6/23/80	12.1	2.8	4.3
4018	7/16/79	47.6	10.1	4.7
	6/9/80	154.5	16.2	9.6
4019	6/9/80	39.3	6.1	6.5
4020	3/11/80	133.4	13.7	9.6

* Application rates were based upon the soil phosphorus requirement.

TABLE 1.5. RATES AND ACRES OF SLUDGE APPLICATION ON MEDINA COUNTY FARMS*

Farm Code	Date of Application	Dry Metric Tons Applied	Hectares	(Dry Metric Tons/Hectare)
1001†	7/7/78	7.3	6.5	1.1
1002‡	10/25/78	8.6	2.0	4.3
	6/28/79	4.5	2.0	2.2
1003	9/5/78	31.7	6.2	5.2
	4/24/79	15.2	5.7	2.7
	11/5/79	15.8	2.4	6.5
	6/17/80	10.0	2.4	4.0
1004‡	5/3/79	12.2	2.6	4.7
	6/4/80	10.5	2.1	4.9
1005†	7/17/78	10.0	8.1	1.3
	9/26/79	4.5	8.1	0.7
1006	6/26/78	5.7	4.8	1.1
	8/21/78	6.3	2.8	2.2
	5/21/79	4.5	2.8	1.6
	7/16/79	6.3	1.6	4.0
	8/15/80	19.0	5.3	3.6
1008	5/23/78	7.3	10.1	0.7
	5/10/79	19.0	10.7	1.8
	8/31/79	5.6	6.1	0.9
	5/6/80	31.7	20.4	1.6
1009	8/9/78	26.3	13.3	2.0
	7/19/79	12.7	13.3	0.9
	5/19/80	22.1	9.3	2.5
1010	9/15/78	5.1	3.2	1.6
	9/5/80	8.2	3.2	2.5
1011†	7/1/80	13.6	5.7	2.5
1012†	9/12/80	9.1	6.1	1.6

* Application rates were based upon the soil phosphorus requirement.
† Farms withdrew from participation during the course of the project.
‡ Farms were started late in the project.

TABLE 1.6. RATES AND ACRES OF SLUDGE APPLICATION ON CLARK COUNTY FARMS*

Farm Code	Date of Application	Dry Metric Tons Applied	Hectares	(Dry Metric Tons/Hectare)
5001	11/19/79	26.1	7.3	3.6
	3/31/81	12.2	3.6	3.4
	1/28/82	15.2	3.2	4.7
5002	10/25/79	23.9	6.7	3.6
	4/1/81	12.0	4.4	2.7
5003	11/8/79	11.0	4.9	2.2
	4/15/81	16.3	3.6	4.5
5004	5/8/80	7.1	1.2	5.8
	4/22/81	19.0	4.0	4.7
5005	12/17/79	35.6	6.9	5.2
	9/19/80	70.2	19.4	3.6
	8/4/81	93.6	19.0	4.9
	2/11/82	13.8	3.2	4.3
5006	2/7/80	17.4	4.8	3.6
	8/20/80	70.2	13.7	5.2
	7/13/81	43.2	11.3	3.8
	2/8/82	7.3	1.6	4.5
5007	4/21/80	31.3	6.1	5.2
	10/20/81	21.8	4.8	4.5
5008	3/2/81	58.0	6.5	9.0
	8/20/81	27.8	3.6	7.6
	11/12/81	32.4	6.9	4.7
5009	3/27/81	19.6	4.8	4.0
5010	8/13/81	53.5	11.9	4.5
5012	12/30/81	14.5	3.2	4.5

* Application rates were based upon the soil phosphorus requirement.

SECTION 2

SLUDGE APPLICATION TO CROPLAND DEMONSTRATION SITES

Terry J. Logan, B.S., M.S., Ph.D.
Robert H. Miller, B.S., M.S., Ph.D.
Richard K. White, B.S., M.S., Ph.D.
D. Lynn Forster, B.S., M.S., Ph.D.
The Ohio State University
Columbus, Ohio 43210

INTRODUCTION

The philosophy of land application of sewage sludge in Ohio is that there is, in most cases, sufficient agricultural land close to the treatment plant to allow application of the sludge at low annual rates (<11 mt/ha). At these rates, there is maximum utilization of the nutrients in sludge and minimum environmental hazard. This section describes the field demonstration studies which were conducted to evaluate the effectiveness of low application rates.

METHODS OF SLUDGE ANALYSIS

pH

Determined directly in liquid sludges. Cake sludge is made into a slurry with distilled water before measurement.

Total Kjeldahl Nitrogen

The microkjeldahl procedure of Bremner (1965) was used. A 5 ml aliquot of liquid sludge was pipeted directly into the Kjeldahl flask. The procedure then followed Bremner (1965). N was determined by steam distillation.

Total Metals and Potassium

The liquid sludges were freeze-dried prior to analysis of metals, phosphorus and potassium. A 0.5 g sample was placed in a pyrex test tube (calibrated to 50 ml volume) on a machined aluminum block which was heated by a Linberg Heavy-Duty hot plate. Five to 10 ml of concentrated HNO_3 was added and small glass funnels were placed in the mouths of the tubes for refluxing. The mixture was slowly brought to 200 C and the volume reduced to about 5 ml. Three ml of concentrated perchloric acid was then added and the mixture digested for 75 minutes. The samples were cooled and the tubes made to volume with double distilled deionized water. They were thoroughly shaken and allowed to stand for 24 hours. For total metals, the supernatant was used directly for analysis by atomic absorption spectroscopy using a Varian Model 375 with background correction and an HP9815A calculator for curve fitting. Metals analyzed include Cd, Cu, Cr, Pb, Ni and Zn. For potassium, four ml of the diluted digest was further diluted to 50 ml and potassium determined by flame emission on the AA.

Phosphorus

A 1-ml aliquot of the diluted digest used for potassium analysis was transferred to a 100-ml volumetric flask. Two drops of 25% 2,4-dinitro phenol were added and then 0.5N NaOH until the color just turned to pale yellow. Ten ml of Murphy-Riley solution, as described by Knudsen (1980), containing 1.5 g L-ascorbic acid/100 ml was added to the volumetric flask and the mixture was diluted to volume with distilled water. After 30 minutes, the absorbance was read on a Beckman Model 24 UV-visible spectrophotometer at 730 nm.

Solids

A sample of sludge containing 50-75 g wet solids was transferred to a beaker, weighed on an analytical balance, oven-dried at 80 C for 48 hours and reweighed.

METHODS OF SOIL ANALYSIS

pH 1:1 in water.

Bray P1 Available P

Knudsen (1980). Absorbance measured at 730 nm. Detection limit 1.0 µg P/g soil.

Total Kjeldahl Nitrogen

Bremner (1965). Digestion with concentrated H_2SO_4 and catalyst ($K_2SO_4/CuSO_4/Se$) on Labconco microkjeldahl digestion apparatus. Neutralized with 10N NaOH and distilled into boric acid. Titrated with 0.01N HCl. Detection limit 100 µg N/g soil.

Total Metals

A 2 g sample of soil was placed in a 100-ml pyrex glass tube in a machined aluminum block on a hot plate in a perchloric acid hood. Five ml of concentrated perchloric acid was added, the tube covered with a small glass funnel for refluxing, and the sample digested for 75 minutes at 200 C. After cooling, the sample was filtered with No. 1 filter paper, brought to 50 ml in a precalibrated test tube, mixed, and metals analyzed by atomic absorption spectroscopy. The six metals (Cd, Cu, Cr, Ni, Pb, Zn) were all analyzed on a Varian Model 375 spectrophotometer with an air-acetylene flame and background correction. Detection limits for the metals were: Cd-0.25; Ni-1.25; Pb-4.0; Cu-2.0; Cr-2.0; Zn-3.0 µg/g soil.

Total Phosphorus

An aliquot of the filtered perchloric acid digest for total metals which was diluted to 50 ml was further diluted 50-100 fold as needed and P was analyzed as ascorbic acid reduced phosphomolybdate at 730 nm. Detection limit 25 µg P/g soil.

Total Potassium

An aliquot of the filtered perchloric acid digest for total metals which was diluted to 50 ml was further diluted 25 fold and K was determined by flame emission on the Varian Model 375 spectrophotometer. Detection limit 200 µg K/g soil.

DTPA Extractable Metals

Soils were extracted with 0.005M DTPA (diethylene triaminepentaacetic acid), 0.1M triethanolamine and 0.01M $CaCl_2$ at pH 7.3 according to Lindsay and Norvell (1978). The extracted metals were analyzed as for total metals. Detection limits were: Cd-0.02; Ni-0.10; Pb-0.32; Cu-0.16; Cr-0.16; Zn-0.24 µg/g soil.

METHODS OF PLANT ANALYSIS

Total Kjeldahl Nitrogen

A 0.2 g sample of ground plant material was digested and analyzed as was described for sludge.

Total Metals

Five grams of ground plant materials was weighed into 250 ml Erlenmeyer flasks. Twenty to thirty ml of concentrated HNO_3 was added and the mixture is allowed to sit at room temperature in a fume hood for at least one hour. Samples were heated to just evaporate the nitric acid over a two-hour period. The samples were kept at low temperature for 8-24 hours until they were lemon colored and then they were cooled and filtered into 20 ml volume calibrated test tubes. Metals were determined directly on the digests by flame AA.

Phosphorus and Potassium

A 0.5 g sample was digested as described for total metals and the digest was then diluted to 100 ml. Further dilutions were made as necessary to get the concentration into the desired range. Phosphorus was determined by ascorbic acid-reduced phosphomolybdate as described for sludge analysis. Potassium was analyzed by flame emission on the AA.

QUALITY ASSURANCE

Quality assurance was maintained in several ways:

1. High purity analytical reagents and commercial standards were used. Double distilled deionized water was used for all metal analyses.
2. All analyses were performed in duplicate together with blanks and a mid-range standard.
3. NBS, USEPA and our own reference samples for sludge, soil and plant analysis were routinely determined.
4. Some sample splitting and analysis was done with other laboratories. We participated in two round-robin methodology checks on sludge and plant analysis with other laboratories in the W-124 Regional Research Committee on Sludge Utilization on Agricultural Land.

STATISTICAL ANALYSIS

All statistical analyses were run with the SAS statistical package at the Ohio State University Computer Center.

SEWAGE TREATMENT PLANT CHARACTERISTICS

Treatment

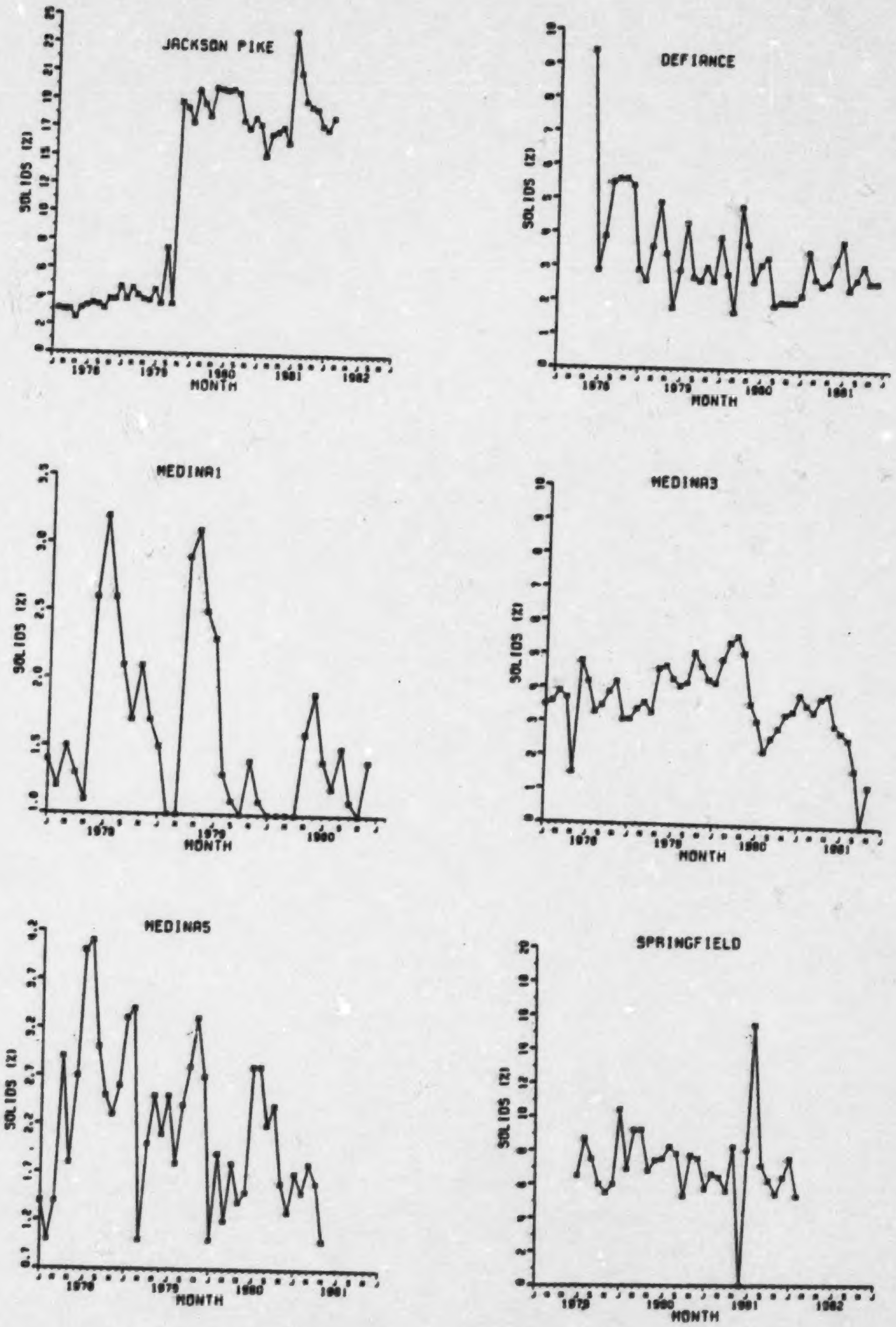
As shown previously (Table 1.1), Columbus, Defiance and Springfield all use anaerobic digestion for sludge stabilization, while the three Medina County plants all have aerobic digestion. A survey of STP's in Ohio in 1980 showed that 55% of the sludge was treated by anaerobic digestion and 38% by aerobic digestion. The Columbus Jackson Pike plant dewaters sludge to 17-20% solids by centrifugation prior to hauling and spreading.

Sludge Quality

Digested sludge was sampled daily at all plants and stored by refrigeration. A composite of the daily samples was analyzed monthly as described in the Methods section. Routine parameters measured included: solids, pH, total kjeldahl nitrogen, NH_3-N, P, K, Cd, Cu, Ni, Pb, Zn and Cr. The results are given in Figures 2.1-2.12 and in Table 2.1.

Figure 2.1 shows that Columbus dewatered its sludge (by centrifugation) and the solids content rose to over 20%. Other than solids, most parameters remained quite steady during the study period. Columbus had the highest Cd content (79.6 µg/g) of the six plants studied.

The Defiance sludge (Figures 2.7-2.12) showed a marked decrease in several of the metals as a result of their industrial pretreatment



JACKSON PIKE

DEFIANCE

MEDINA1

MEDINA3

MEDINA5

SPRINGFIELD

Figure 2.1. Solids content of the six sludges studied (1978-1982).

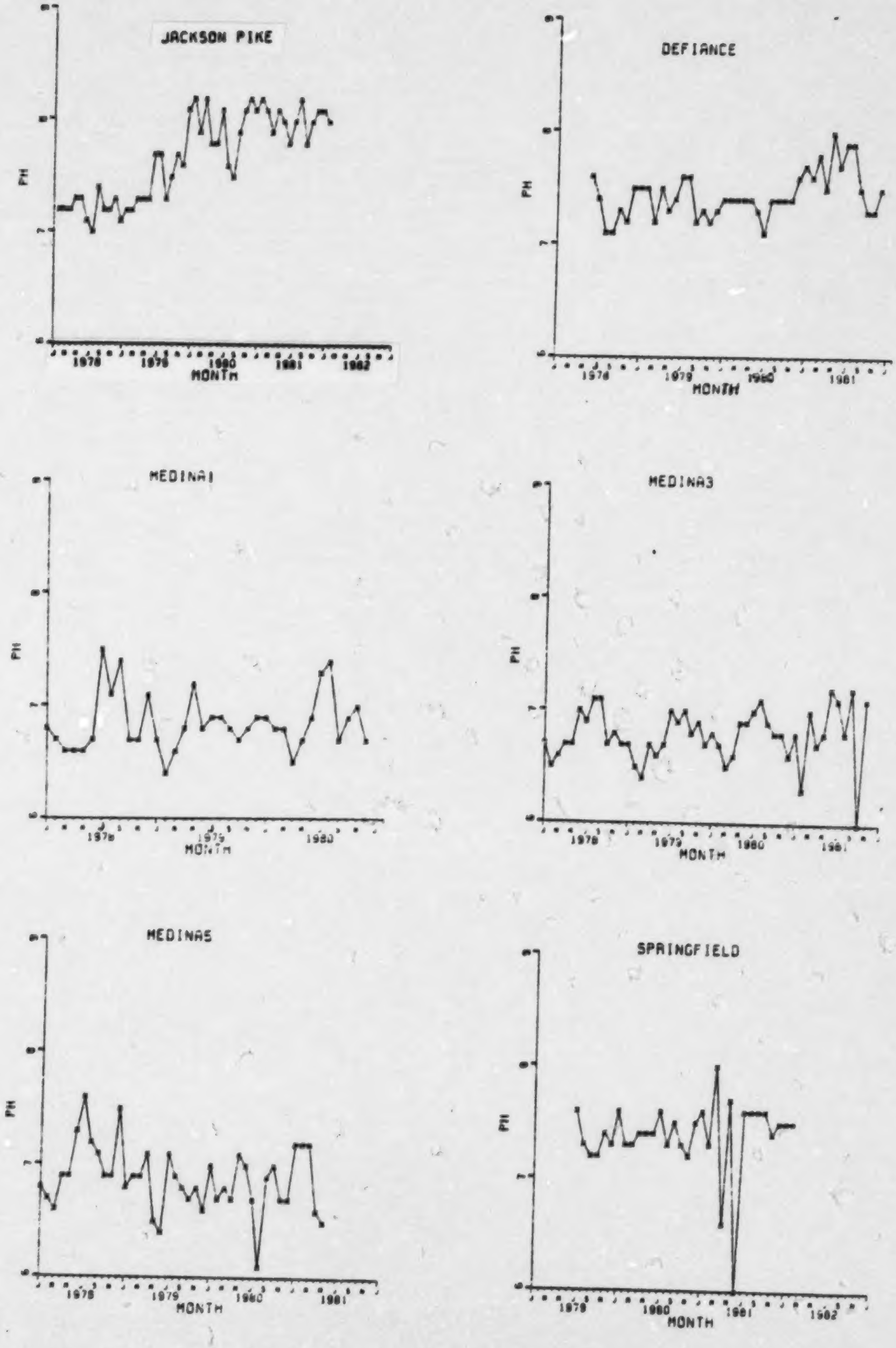


Figure 2.2. Acidity (pH) of the six sludges studied (1978-1982).

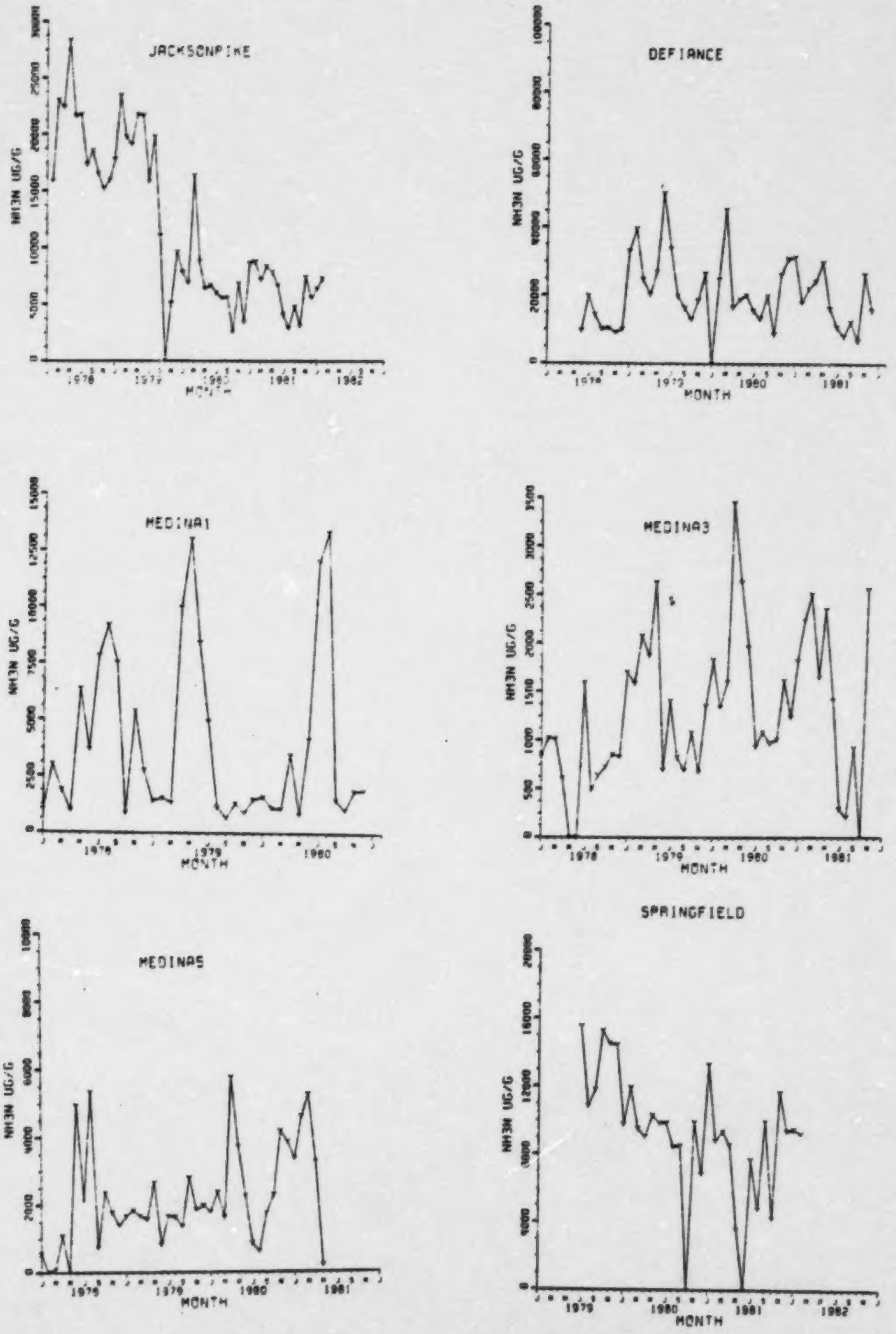


Figure 2.3. Ammonia content of the six sludges studied (1978-1982).

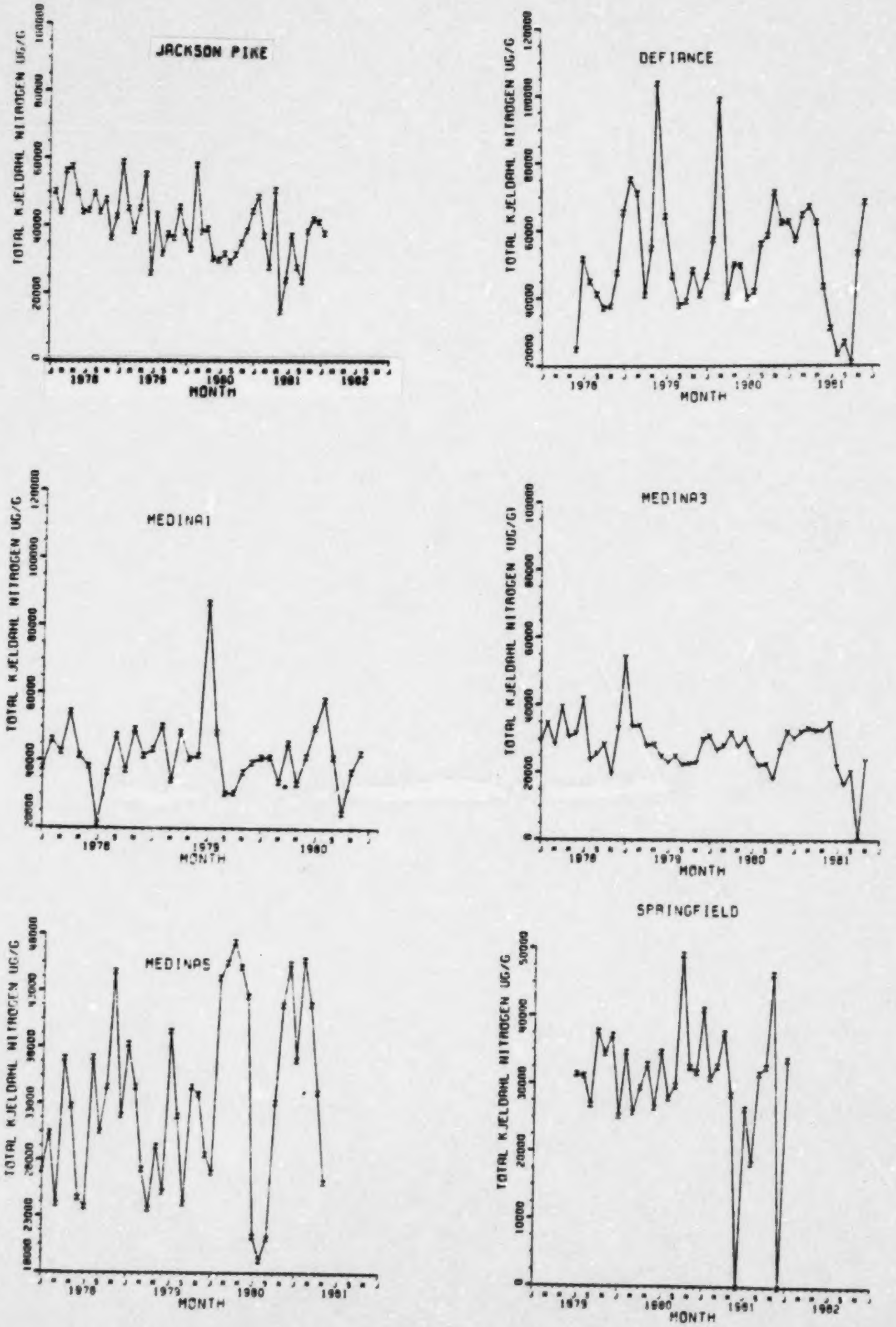


Figure 2.4. Total Kjeldahl content of the six sludges studied (1978-1982).

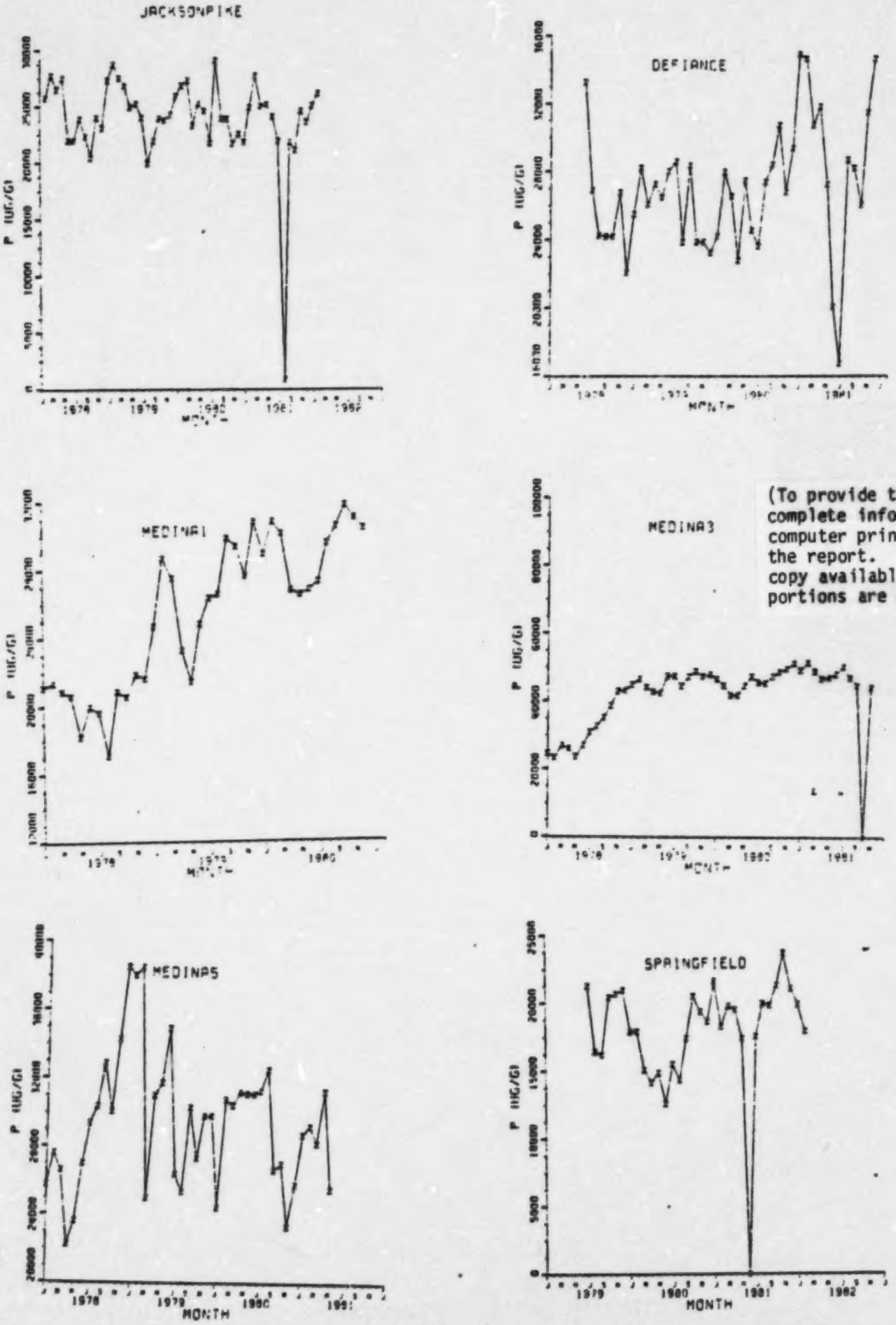


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Figure 2.5. Phosphorus content of the six sludges studied (1978-1982).

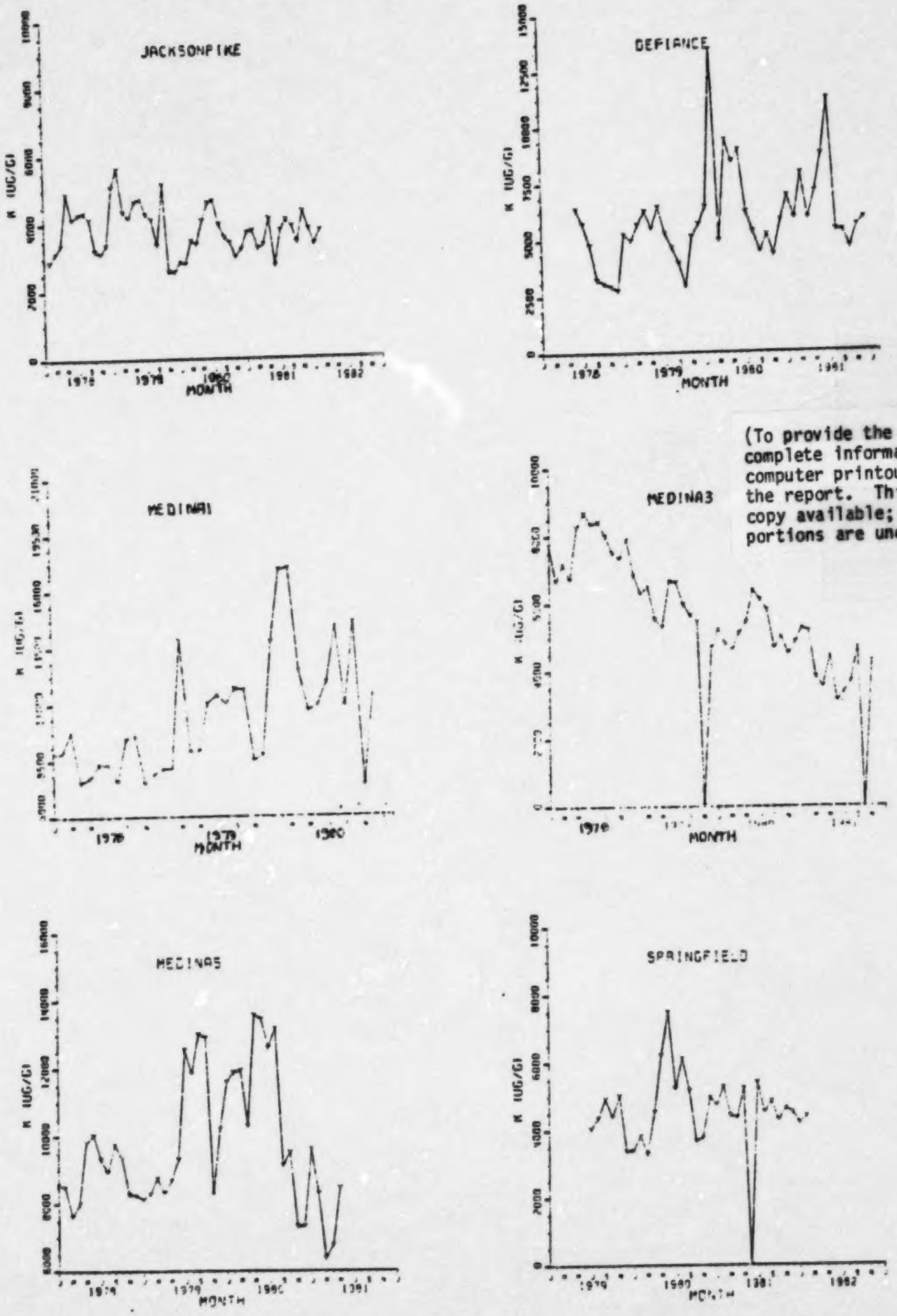

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Figure 2.6. Potassium content of the six sludges studied (1978-1982).

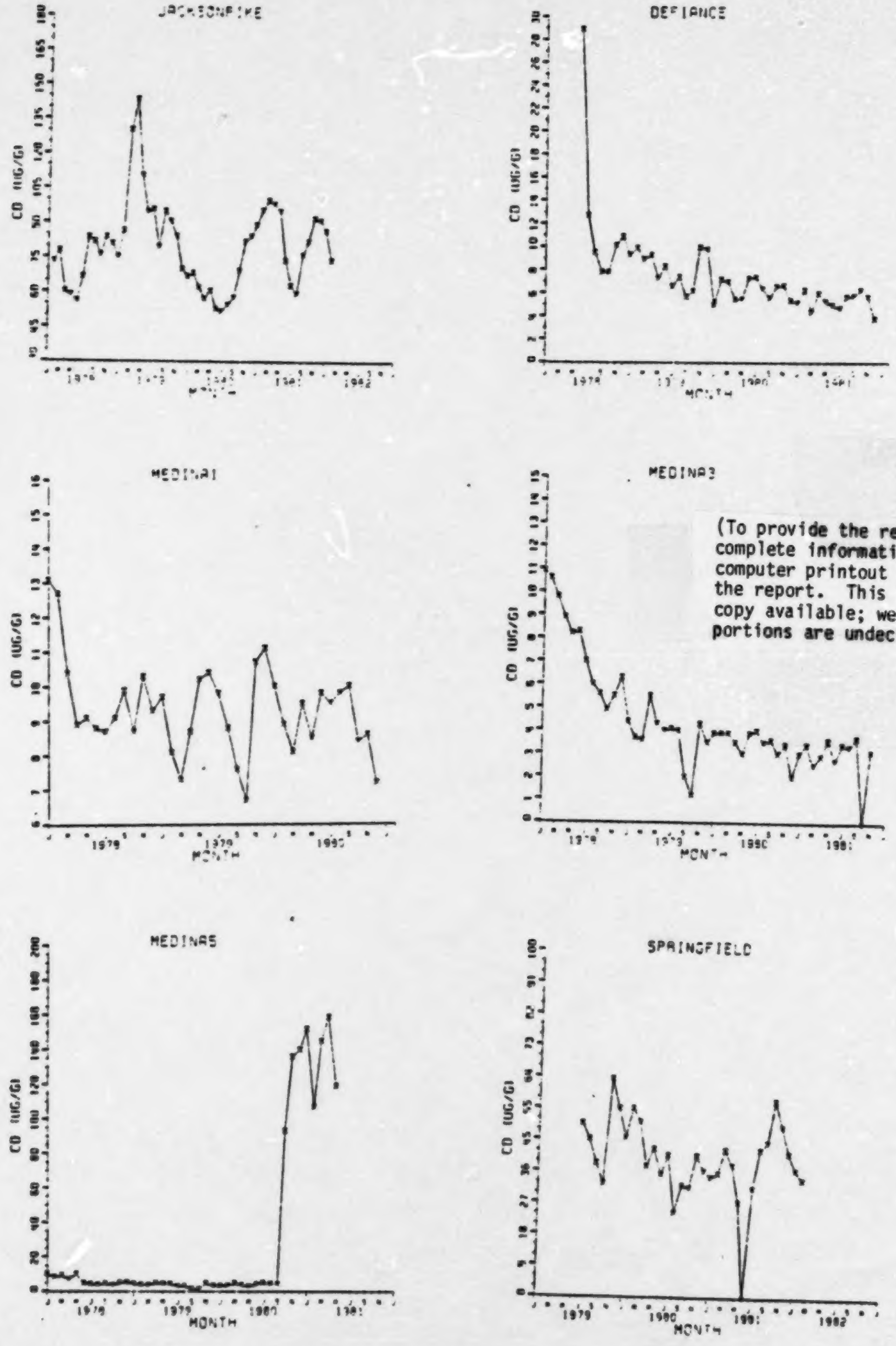

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Figure 2.7. Cadmium content of the six sludges studied (1978-1982).

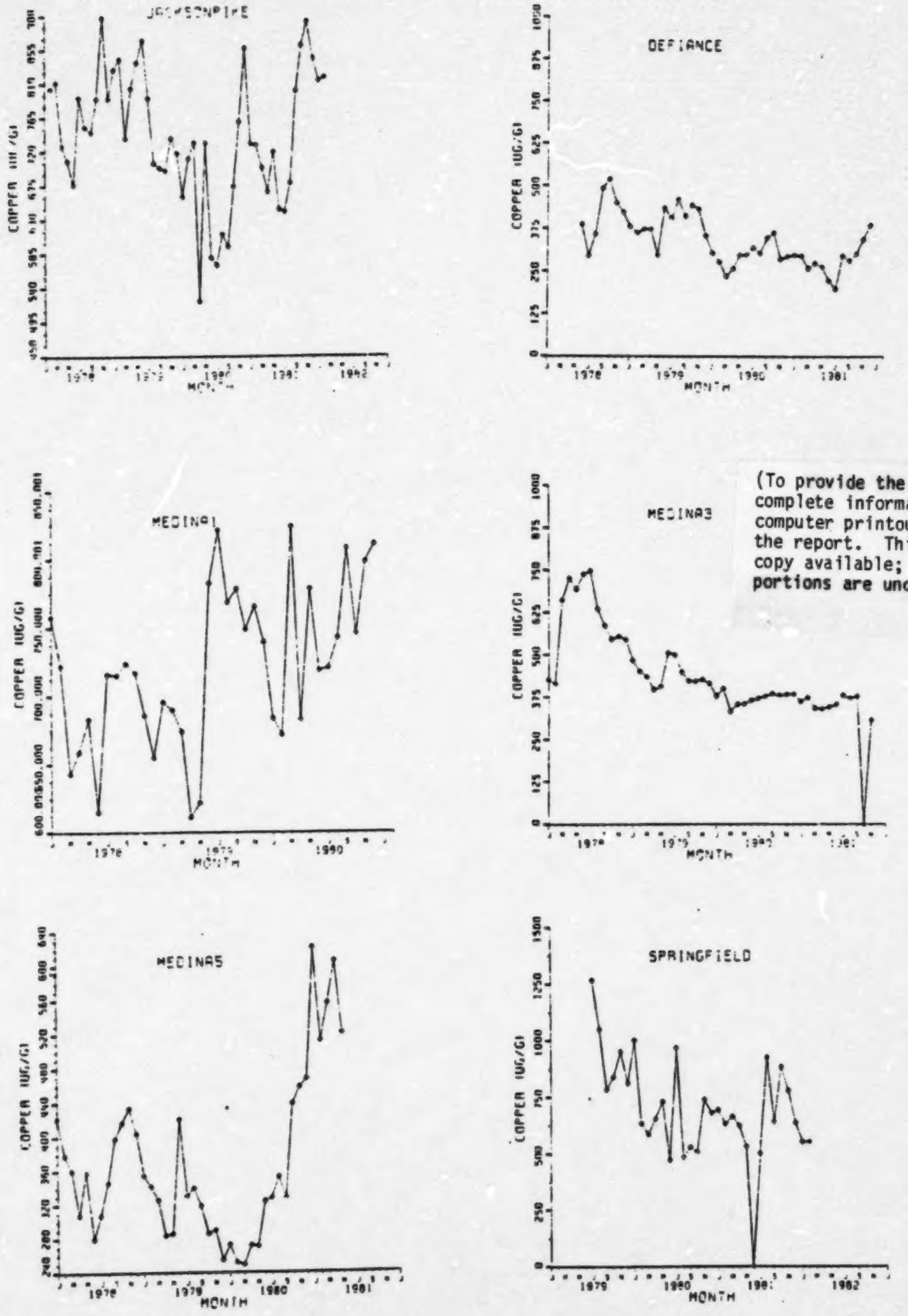

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Figure 2.8. Copper content of the six sludges studied (1978-1982).

(To provide the reader with complete information, this computer printout is included in the report. This is the best copy available; we regret that portions are undecipherable.)

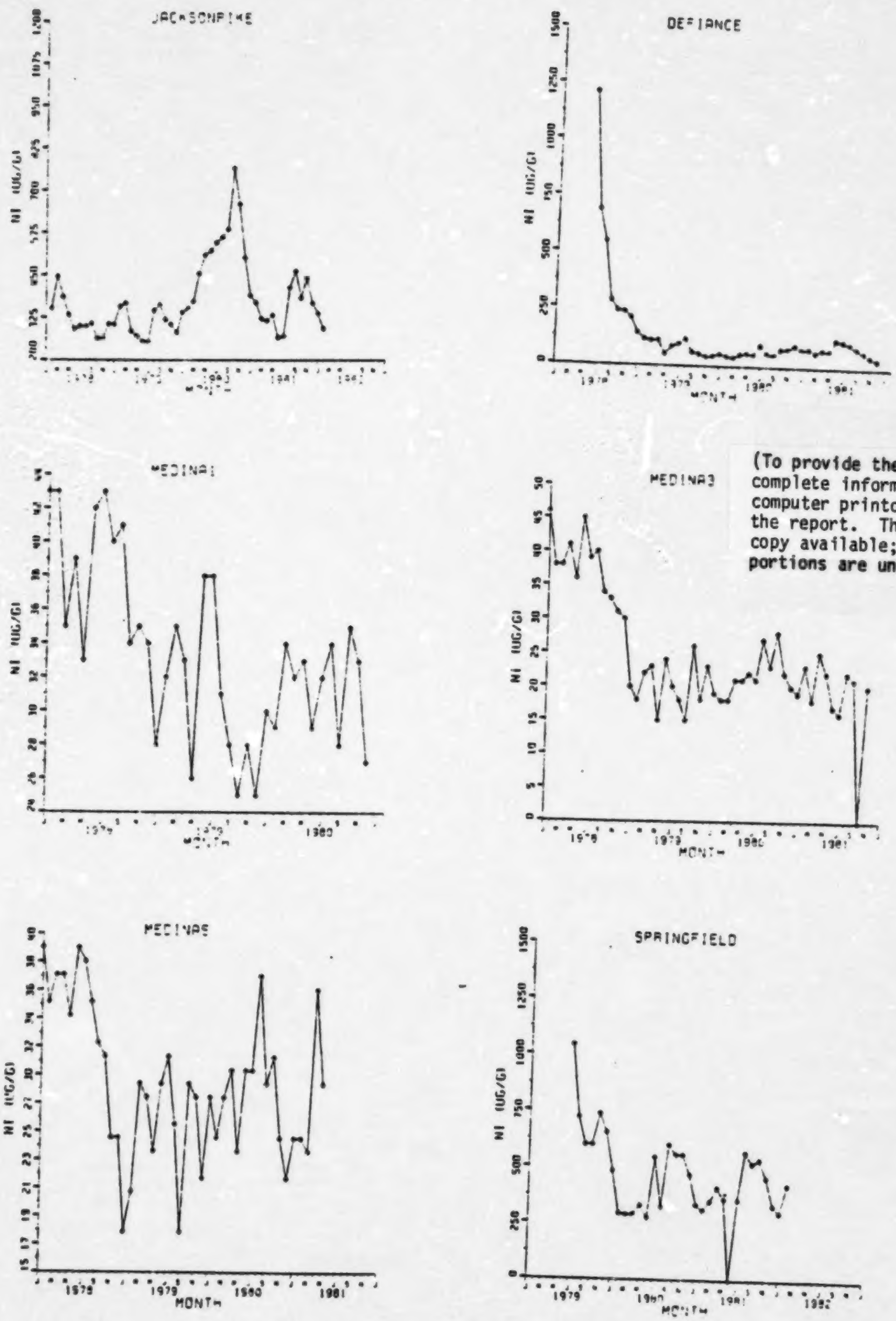

Figure 2.9. Nickel content of the six sludges studied (1978-1982).

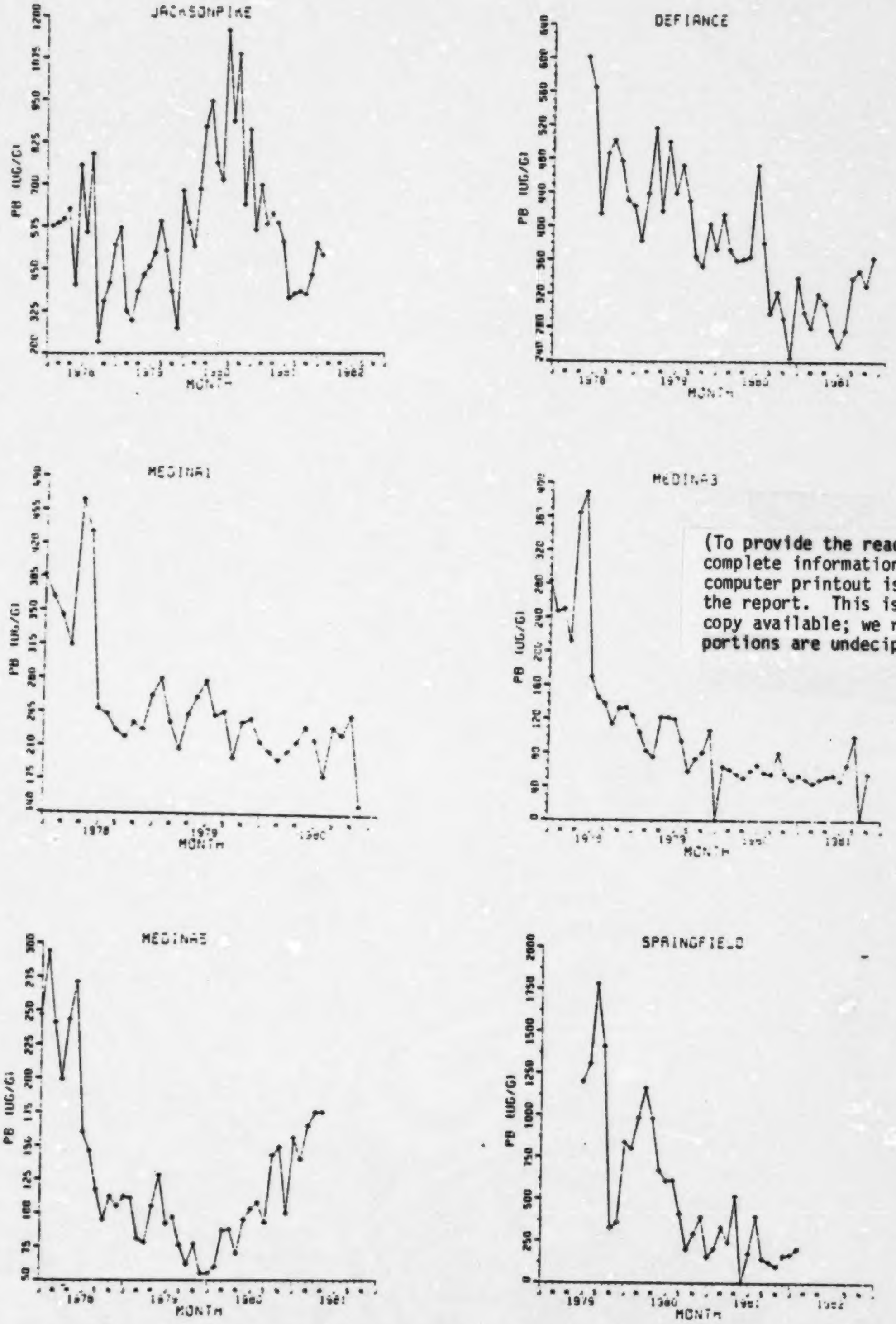

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Figure 2.10. Lead content of the six sludges studied (1978-1982).

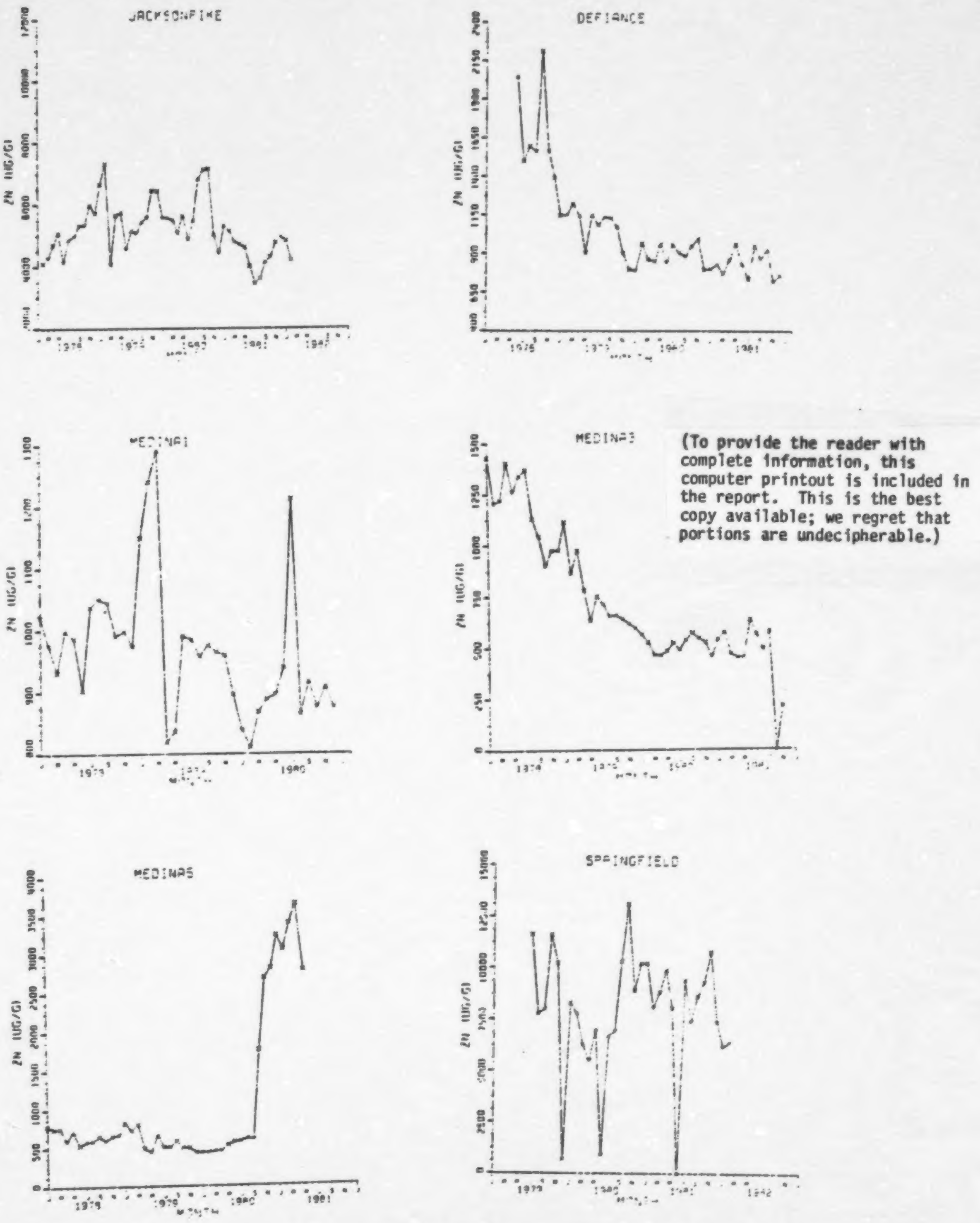

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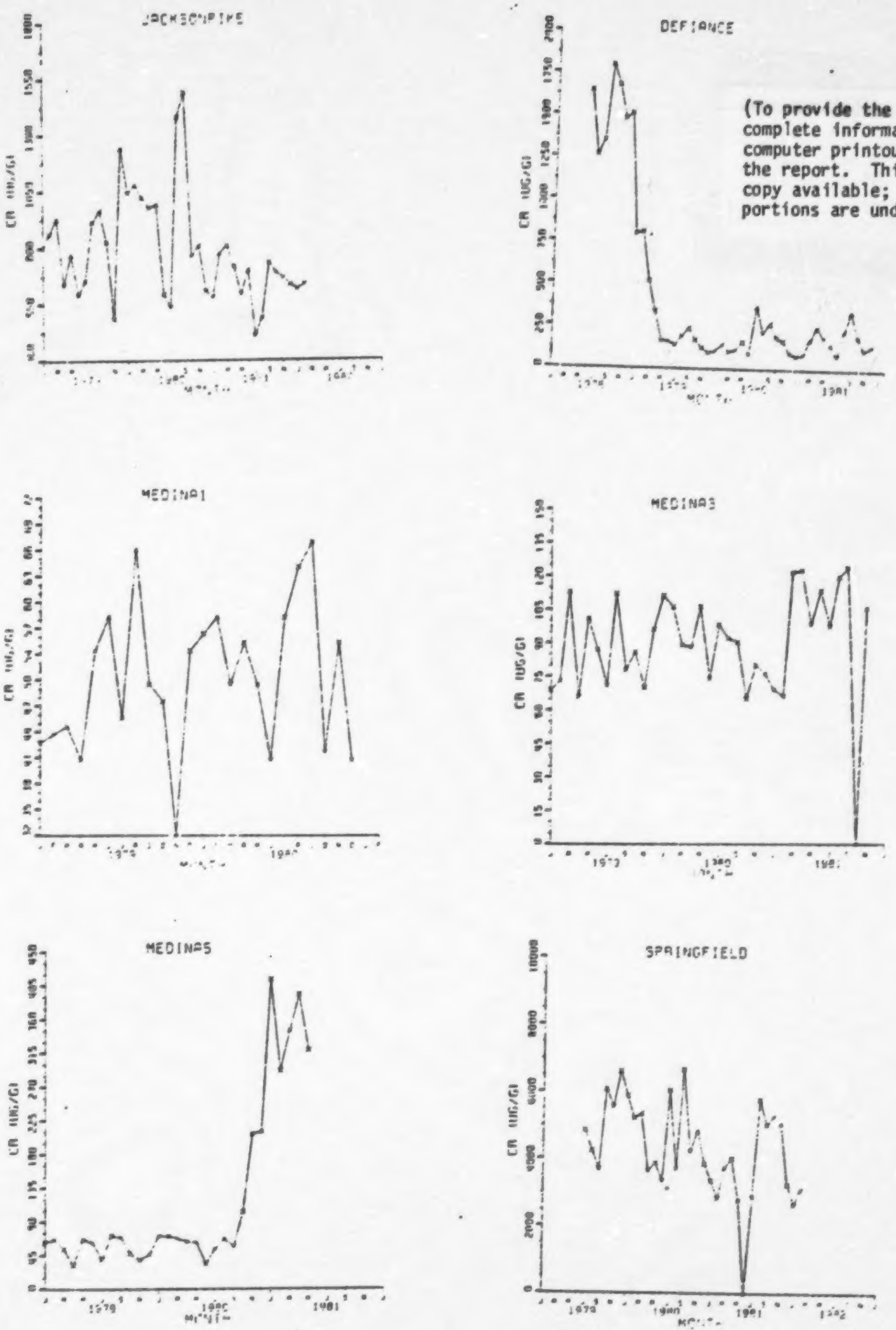
Figure 2.11. Zinc content of the six sludges studied (1978-1982).



(To provide the reader with complete information, this computer printout is included in the report. This is the best copy available; we regret that portions are undecipherable.)

Figure 2.12. Chromium content of the six sludges studied (1978-1982).

TABLE 2.1. RANGES AND MEANS OF PARAMETERS FOR SLUDGES ANALYZED MONTHLY DURING 1978-1982

Statistic	pH	Solids	TKN	NH_3-N	P	K	Cu	Cd	Pb	Ni	Zn	Cr
		%					µg/g					
						Columbus						
Maximum	8.2	24.0†	5.89	2.83	2.90	0.56	898	143.4	1159	766	7316	193
Minimum	7.0	2.6†	1.49	0.25	1.99	0.26	523	51.7	234	253	3439	3
Mean	7.7	12.1	4.01	1.19	2.43	0.38	741	79.6	572	375	5181	109
C.V.*	5.1	--†	24.1	59.2	9.2	18.7	11.8	23.6	35.4	29.5	17.5	64.2
						Defiance						
Maximum	8.0	9.4	10.36	5.00	3.49	1.34	520	28.9	601	1212	2213	255
Minimum	7.1	1.7	2.09	0.68	1.67	0.28	198	3.8	244	6	721	5
Mean	7.4	3.3	5.18	2.07	2.70	0.59	340	7.6	386	143	1058	102
C.V.	2.8	42.4	33.8	48.7	14.0	35.9	22.0	50.9	21.6	146	31.4	84.5
						Medina 100						
Maximum	7.5	3.2	8.69	1.33	3.17	1.70	825	13.1	465	43	1292	236
Minimum	6.4	0.6	2.12	0.06	1.71	0.74	611	6.7	149	25	810	87
Mean	6.9	1.6	4.22	0.39	2.54	1.04	722	9.3	254	33	969	151
C.V.	3.8	43.0	26.1	97.0	17.0	25.1	8.1	14.3	26.7	15.8	11.7	39.5
						Medina 300						
Maximum	7.2	5.6	5.41	0.34	5.14	0.87	820	10.9	309	46	1430	267
Minimum	6.3	1.2	1.66	0.02	2.34	0.31	306	1.2	44	15	212	40
Mean	6.8	3.7	2.86	0.14	4.26	0.59	462	4.6	110	25	745	175
C.V.	3.1	26.2	23.1	52.1	18.7	24.8	29.1	49.2	74.4	33.1	42.1	49.5
						Medina 500						
Maximum	7.6	4.1	4.74	0.57	3.85	1.36	622	159	294	39	3644	222
Minimum	6.1	0.6	1.38	0.01	2.23	0.64	249	1.3	55	18	435	.99
Mean	6.9	2.1	3.32	0.23	2.94	0.97	368	29.4	128	29	1048	153
C.V.	4.2	38.9	25.7	63.7	13.5	20.5	25.9	176	47.3	19.3	92.8	33.9
						Springfield						
Maximum	8.0	15.4	4.92	1.55	2.35	0.75	1268	63.1	1778	1043	13117	223
Minimum	6.6	5.3	1.84	0.36	1.25	0.33	473	24.6	98	278	5493	1
Mean	7.4	7.4	3.29	0.99	1.84	0.47	719	41.3	556	473	8595	75
C.V.	3.1	26.6	20.4	29.5	14.1	18.9	26.7	21.6	80.4	36.5	21.9	74.8

* Coefficient of variation.
† Includes liquid and centrifuged sludges.

program. Nickel, in particular, was greatly reduced. The present metal levels make this a fairly "clean" sludge.

The Springfield sludge is high in solids for a liquid sludge (7.4%) and had the highest Zn content (8595 µg/g) of the six plants.

The Medina sludges were all low in solids and, as is characteristic of aerobic digestion, they were low in NH_3-N compared to the anaerobic sludges. These treatment plants serve primarily rural and suburban residential areas, and the metals are therefore quite low. However, the City of Medina was connected to the Medina 500 treatment plant in 1980, and Figure 2.7 shows that the Cd content increased dramatically.

In addition to the low NH_3-N, the aerobic sludges had slightly lower pH's than the anaerobic sludges.

The variations in sludge parameters shown in Figures 2.1-2.12 indicate that some sludge constituents do not change rapidly (e.g. phosphate) while others like NH_3-N and TKN are quite variable. Repeated, systematic sludge analysis is necessary to document variations and trends in sludge quality. The daily composited, monthly analyzed sample is probably adequate for smaller treatment plants with limited industrial discharges, while more frequent analysis of key parameters (e.g. NH_3-N, Cd) may be needed for larger plants with a wide variety of discharges.

The main agricultural benefit of digested sewage sludge in agricultural production is its content of available nutrients. These are given in Table 2.2 for the six treatment plants in the study. All of the ammonia-N is assumed to be available and 30% of the organic-N is assumed to be available in the year it is applied. We also assumed that there would be no residual availability of organic-N after the first year. All of the phosphorus and potassium were assumed to be available. It can be seen that phosphorus contributes most of the total nutrient value of sludge, but this may not be entirely correct, since all of the phosphorus is probably not available and part of it is irreversibly tied up by the soil. Potassium contributes very little to the total nutrient value of sludge. The calculated value of sludge does not include the micronutrients or the organic matter.

USEPA regulates the annual and cumulative applications of cadmium in sewage sludge (EPA, 1979) and Ohio regulates the cumulative applications of Ni, Cu, Pb and Zn (Ohio Sludge Guide, 1982). Table 2.3 indicates the annual Cd loading at a sludge application rate of 11.5 mt/ha (a typical rate for supplying nitrogen to corn), the maximum cumulative sludge application and the limiting metal. Only Columbus would violate Federal regulations on annual Cd loads, and only after 1987. Zinc in the Springfield and Columbus sludges would limit sludge applications to 58 and 97 metric tons/hectare, respectively, which would give a useful spreading lifetime of a particular site of 5 and 8 years for those sludges, at an annual application rate of 11.5 mt/ha. The

TABLE 2.2. THE AVERAGE AVAILABLE NUTRIENTS AND THEIR VALUE IN THE SIX SLUDGES STUDIED

Sludge	Nitrogen*		Phosphorus*		Potassium*		Total Value
	kg/mt	$/mt†	kg/mt	$/mt†	kg/mt	$/mt†	$/mt
Columbus	20.4	11.22	24.3	30.62	3.8	1.01	42.83
Defiance	30.0	16.50	27.0	34.02	5.9	1.57	52.09
Medina 100	15.4	8.47	25.4	32.00	10.4	2.77	43.24
Medina 300	9.6	5.28	42.6	53.68	5.9	1.57	60.53
Medina 500	11.6	6.38	29.4	37.04	9.7	2.58	46.00
Springfield	16.8	9.24	18.4	23.18	4.7	1.25	33.67

* Assumes all of the NH_3-N and 30% of the organic N TKN-(NH_3-N) is available. Assumes all of the P and K are available.

† Nitrogen - $0.55/kg; P - $1.26/kg; K - $0.27/kg.

TABLE 2.3. ANNUAL CADMIUM LOADINGS AND ALLOWABLE SLUDGE LOADINGS BASED ON METAL ACCUMULATIONS

Sludge	Cd Loading* (kg/ha/yr) at Sludge Application of 11.5 mt/ha	Cumulative Metal Loading Limitation†	
		Limiting Metal	Maximum Sludge Loading (mtons)
Columbus	0.92	Zn	97
Defiance	0.09	Zn	473
Medina 100	0.11	Cu	346
Medina 300	0.05	Cu	541
Medina 500	0.38	Cd	340
Springfield	0.47	Zn	58

* Allowable annual Cd loading is 2.0 kg/ha/yr (present to June 30, 1984); 1.25 kg/ha/yr (July 1, 1984 — Dec. 31, 1986); 0.5 kg/ha/yr (after Jan. 1, 1987). (EPA, 1979).

† Assumes soil cation exchange capacity of 5-15 meq/100g, and maximum cumulative metals (kg/ha) of Cd-10, Cu-250, Ni-250, Zn-500 and Pb-1000 (Ohio Sludge Guide, 1982).

other four sludges could be safely applied for much longer periods of time.

Landspreading Methods

Columbus--

The land application program for the City of Columbus' Jackson Pike sewage treatment plant was concentrated in southern Franklin and northern Pickaway Counties, with smaller amounts of sludge spread in Madison County (Figure 1.1). Most of the sites were within 25 km for the demonstrations, and Columbus has planned to keep future landspreading within this radius. In designing the program, Columbus decided that it would be cost-effective to dewater the sludge prior to transporting it to the application site to reduce hauling costs. The liquid sludge is removed from the anaerobic digestor and dewatered by centrifugation to 17-20% solids. Sludge is dewatered just prior to its transport to the field.

S and L Fertilizer Company of White House, Ohio was the contractor to Columbus for the hauling and spreading. The City provided a full-time agronomist to identify sites, secure owner or operator approval, obtain necessary permits from Ohio EPA and to monitor the program. Sludge was hauled from the treatment plant to the application site in open-top, tractor-trailer trucks each with a capacity of 25 wet metric tons (Figure 2.13). The trailers were specially designed with wide lips on the tops and baffles to prevent the load from sliding and with water-tight seals on the tail gate. The load is dumped on the field (Figure 2.14) at the spreading site on a pad prepared by laying gravel. This is to protect the area from compaction and rutting during the hauling and spreading operations. The stockpiled sludge is loaded into the spreader with a front-end loader (Figure 2.15) equipped with flotation tires and a non-skid rear end to minimize compaction and rutting. Thirty loads of sludge per week are normally hauled to the site and stockpiled. This is then spread in one day. The spreader (Figure 2.16) is a specially designed box on an Ag-Chem Terragator five-wheel truck with flotation tires. The box has a capacity of 13 wet metric tons and can spread 4.5 to 15 dry metric tons/hectare with a single pass. The box is fitted with chains to move the sludge to the rear, and the gear-driven beaters have several speeds to aid in controlling the spreading pattern. The box is also heated to allow winter application.

The Columbus operation was designed for year-round spreading, including winter spreading. However, periods of snowmelt or rain were avoided to prevent surface runoff.

Medina--

The land application program in Medina County had just been initiated when it became part of this study. Medina has a county-wide system of small treatment plants and package plants which serve the urban and suburban communities in the County. Until August of 1980, the City of Medina had its own treatment plant, but this was shut down and

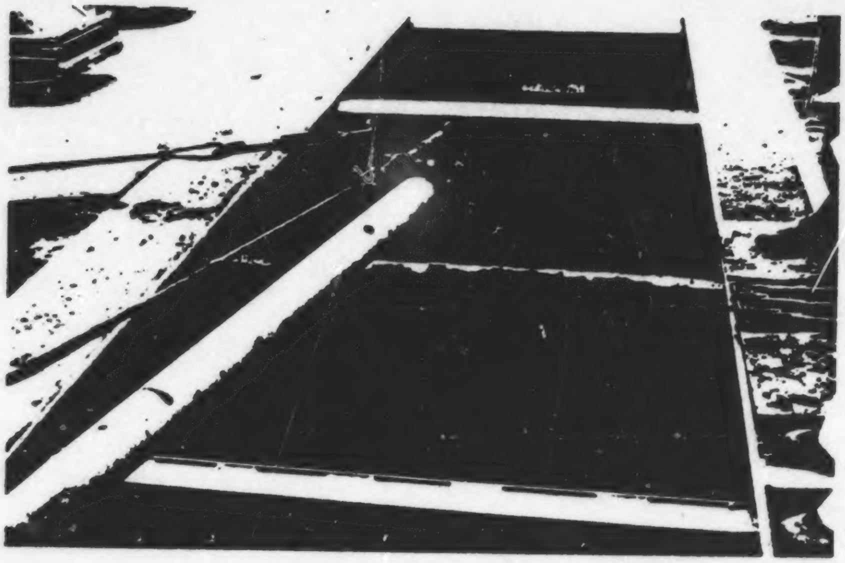


Figure 2.13. Top view of haul truck for Columbus cake sludge.

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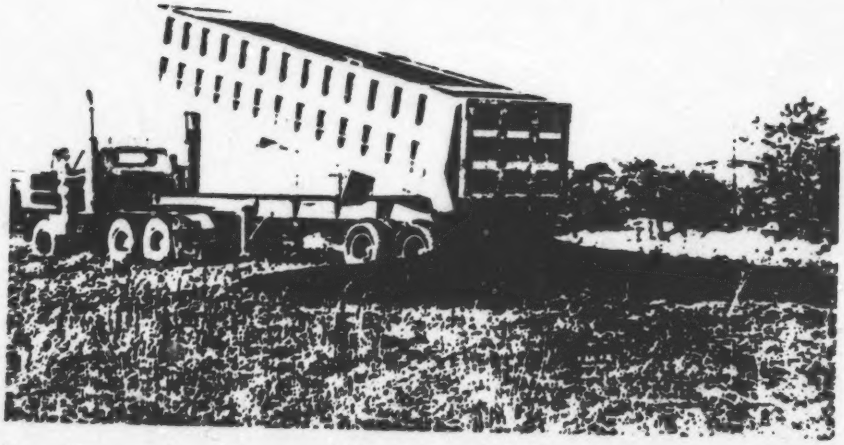

Figure 2.14. Haul truck unloading Columbus cake sludge at application site.

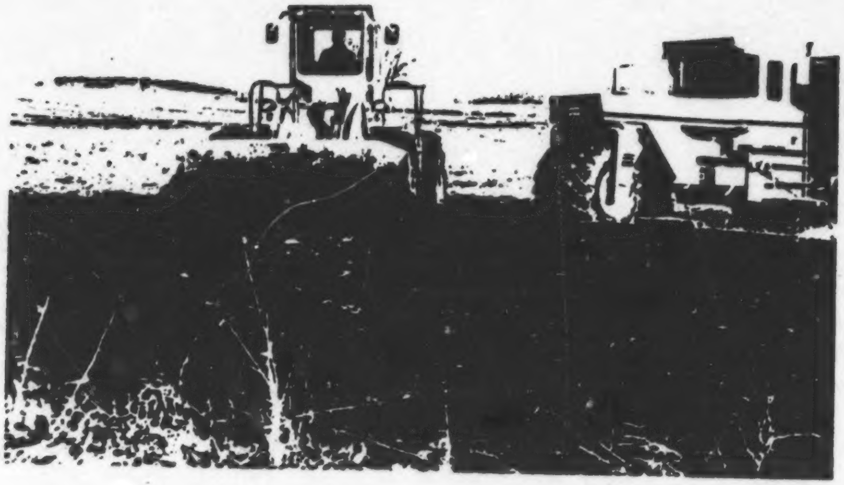

Figure 2.15. Front-end loader used to transfer Columbus cake sludge to applicator vehicle.

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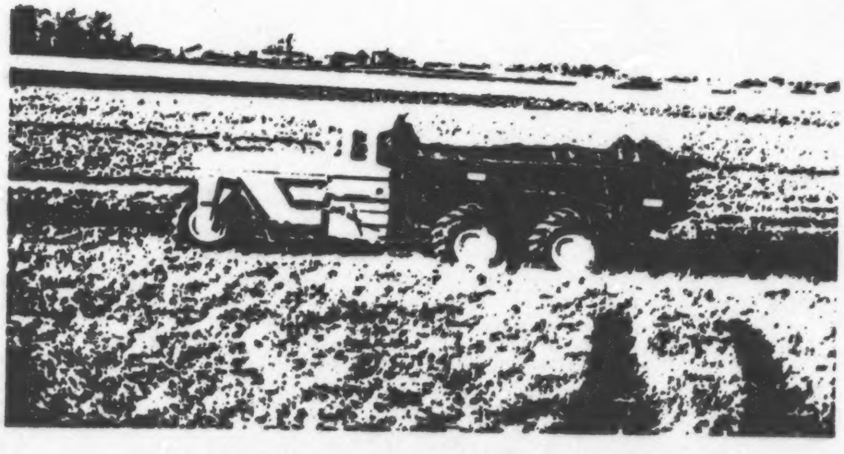

Figure 2.16. Ag-Chem Terragator® with custom box used to spread Columbus cake sludge.

the City connected to the County system. Sludges from three of the County treatment plants were used in this system: Medina 100, 300 and 500.

Liquid digested sludge was hauled to the application site in two 22,800 liter tankers. Sludge was pumped directly into the spreader; therefore, sludge was hauled only during spreading periods.

Sludge was spread with a locally custom-built tank truck (Figure 2.17). It has a capacity of 7600 liters and is vacuum filled and pressure discharged. It is equipped with flotation tires to minimize compaction. Because of the low solids contents of the Medina sludges (Table 2.1), single pass applications are low (<2 mt/ha). However, low application rates were generally used in the Medina program because of the high availability of land compared to the volume of sludge produced.

The County provided a full-time employee to handle site selections and approvals, schedule sludge applications, acquire necessary permits, monitor the sites and keep records. As in the Columbus project, a full-time employee to coordinate the land application program is a critical component of any successful program.

Defiance--

This City has been land spreading sludge for many years on its own property adjacent to the treatment plant. High application rates have been used to handle the sludge produced on the available City land. This has resulted in accumulations of heavy metals that are approaching the maximum allowable in Ohio. In particular, Cd levels in the soil are near the maximum allowable according to Federal regulations (EPA, 1979), and this area will have to be managed as a dedicated site in the future. Some sludge is dried on sand beds and given away.

Until 1979, the City used a liquid manure spreader (11,400 liters) and a farm tractor to spread liquid digested sludge on their own land (Figure 2.18). This equipment, however, is inadequate for off-site hauling and spreading. In 1979, Defiance purchased a 7,600 liter commercial tank truck with flotation tires (Figure 2.19). This vehicle is used to haul and spread sludge on farmland within 5 km of the plant. Fortunately, there is a large amount of agricultural land within this radius. Because of wet soils in the area, spreading on farmland is limited to the period June-February (excluding periods of winter rain or snowmelt). Because of the limited amount of sludge spread off-site, the land application program is managed by the treatment plant. Also, a local farmer has a contract to farm the City's land. The City should increase the amount of sludge spread off-site, and reserve its own land as a back-up. However, the treatment plant is unwilling to do so until the City can purchase a nurse truck to haul sludge to the field. There is considerable tire wear to the spreader when it is used to haul sludge to sites away from the plant, and there is little incentive to the City to expand off-site land application unless it would be forced to manage its own property as a dedicated site.

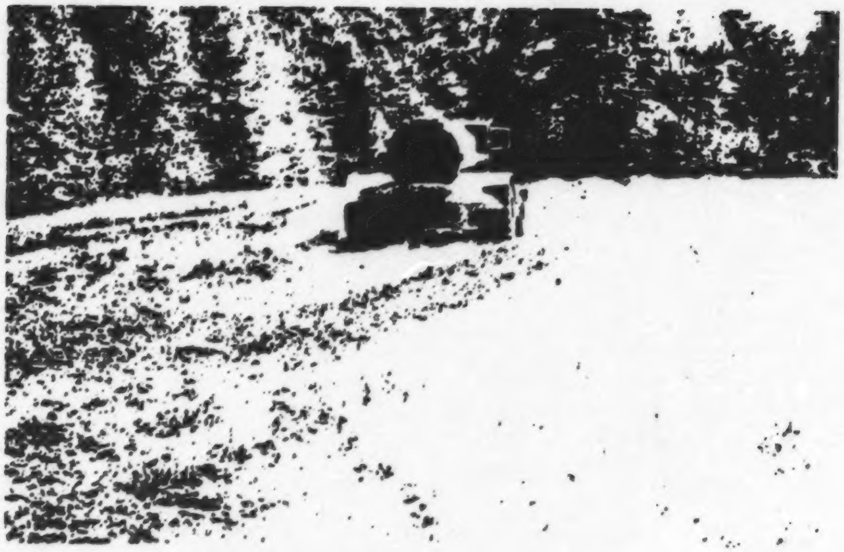

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Figure 2.17. Liquid sludge tank truck used to apply Medina County sludge.

Figure 2.18. Liquid manure spreader and tractor used to apply Defiance liquid sludge.

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Figure 2.19. Liquid sludge tank truck used to apply Defiance liquid sludge.

Springfield--

Springfield has had a limited land application program in Clark County for several years prior to this project. The City uses two tank trucks (11,400 liters) to haul and spread sludge (Figure 2.20). These are gravity flow and have tandem axles. Because they are not equipped with flotation tires, soil compaction and rutting are problems, and these vehicles are easily stuck in wet fields. In addition, the lack of separate nurse trucks for sludge hauling greatly limit the potential for land application of Springfield's sludge.

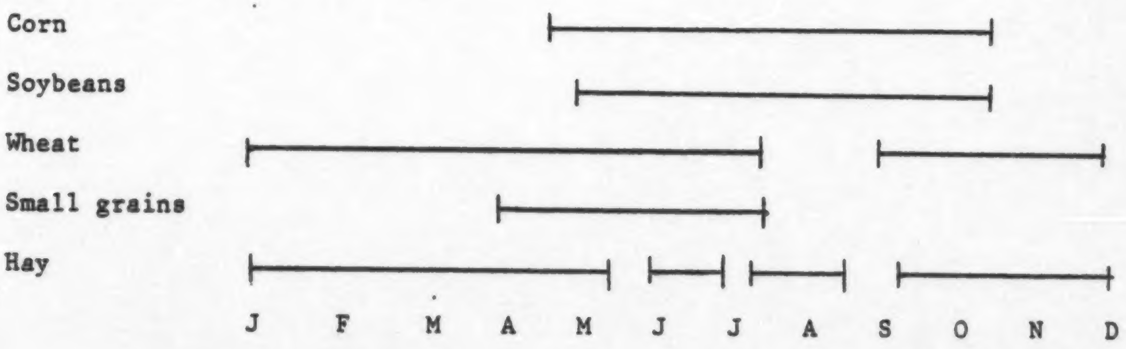
The land application program is run by the sewage treatment plant with agronomic assistance provided by the local county agent. The future success of land application for Springfield will require the purchase of a modern sludge spreader and one or more nurse trucks.

SOILS AND CROPS

Columbus

Columbus sludge is primarily spread in southern Franklin and northern Pickaway Counties with smaller quantities in Madison County. This general area is on the southern edge of the glaciated region of Ohio. The soils are developed in Wisconsin age (15-18 thousand years) glacial till and loess with some soils formed in glacial outwash. These soils are flat to gently sloping and their major limitations for agricultural use are wetness and erosion. Medium textures are common, permeabilities are moderate to slow and seasonal high water tables are common. The soils are naturally fertile, have medium to high organic matter contents and are slightly acid. Specific chemical properties will be given in a later section. The major limitations to sludge application are seasonal wetness and localized steep slopes.

The till plain region of Ohio is primarily used for mixed grain farming with corn and soybeans the major crops. There are smaller acreages of wheat, other small grains, hay fields and pastures. During the demonstration project, sludge was applied to all major crops, and the major limitation to sludge application to specific crops is timing. The following indicates the periods or "windows" that are available for sludge spreading in the North Central region of Ohio (the lines are the periods when the crop is growing):



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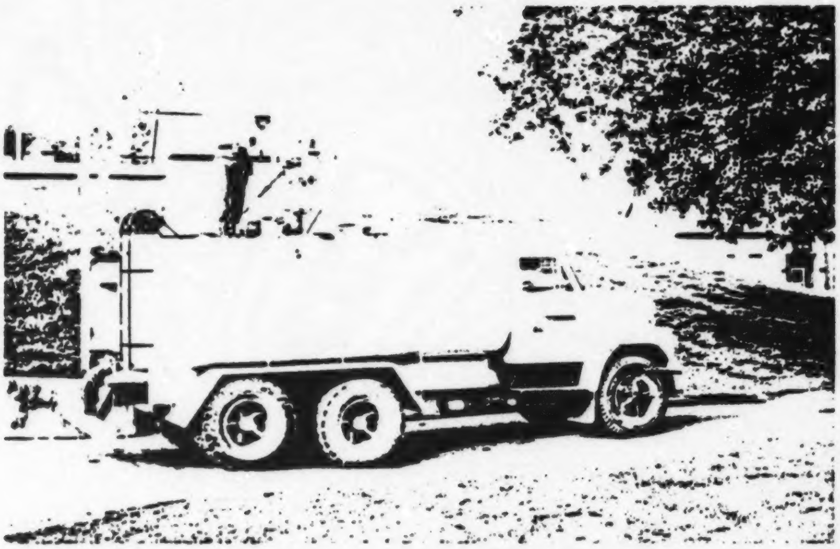


Figure 2.20. Tandem axle liquid sludge tank truck used to apply Springfield liquid sludge.

In the case of hay or pasture crops, the Ohio Sludge Guide (1982) recommends that sludge only be applied just after the hay has been cut or grazed. This is to prevent direct ingestion by livestock of sludge adhering to the vegetation.

Medina

Medina County sludge is spread entirely within the County. This area is in the eastern lobe of the Wisconsin till plain. The soils tend to be more acid, have lower base saturation, be somewhat steeper and somewhat less fertile than the soils previously described in the Columbus area. The greatest limitations to sludge application are seasonal wetness and steep slopes.

This area of Ohio is mixed grain farming (corn, soybeans, wheat and other small grains) and dairy farming (hay and pasture land). There is a greater opportunity to use sludge on hay and pasture land in Medina County than in the other three project areas.

Defiance

All of the sludge from the City of Defiance is spread within Defiance County. This area is located in the Lake Plain region of Northwestern Ohio (Figure 1.1) and the soils are derived from recent (~ 8,000 years) lacustrine calcareous sediments. The soils are level, poorly drained, fine-textured, with high pH's, high base saturations and high CEC's. The major limitation to sludge spreading is prolonged seasonal wetness.

The Lake Plain region is intensely farmed with corn, soybeans and wheat the major crops. There are only small acreages of other small grains and forage crops.

Springfield

The City of Springfield spreads all of its sludge in Clark County (Figure 1.1) in west-central Ohio. The soils are derived from Wisconsin age glacial till, loess and outwash gravel. The soils are flat to steeply sloping and have properties that are similar to those in the Columbus project area. Seasonal wetness and steep slopes are the major limitations to sludge landspreading. The crops in the area are primarily corn, soybeans and wheat with some hay and pasture land and small amounts of other small grains.

THE DEMONSTRATION SITES

Methods

In each study area, several plots were established on farmer fields to determine yield and metal uptake from sludge applications. These were designed more as demonstrations rather than experiments and were not replicated. The standard design was to apply sludge at an agronomic rate or rates for a particular crop and compare crop yield and metal uptake with a control plot in the same field. The control was usually a recommended rate of fertilizer for that crop, or a zero fertilizer "check"; in a few instances there were both fertilizer and check plots. Plot sizes varied from as small as 0.01 ha to several hectares. Soil samples were taken before sludge application and analyzed for pH, lime test index, exchangeable bases, cation exchange capacity, Bray P1 available P and total metals (Cd, Cu, Ni, Pb, Zn and Cr). At the end of the study, a random selection of the sites were sampled and the soils reanalyzed for total metals. Sludge loading rates were estimated from volume applied and solids content. Plant leaf tissue was sampled at tasseling (corn), flowering (soybeans), heading (wheat and oats) or prior to cutting (hay) and analyzed for N, P, K and metals. At harvest, yields were measured by hand by sampling 10-30 meters of row and weighing. Grain was also sampled at harvest for N, P, K and metal analysis.

Background Soil Fertility

Table 2.4 summarizes the pH, Bray P1 available P, exchangeable potassium and cation exchange capacity data for the demonstration sites in each project area. The results show that there were very few differences between the four project areas compared to differences among soils in each project area. Soil pH's tend to be slightly acid and available phosphorus and potassium are both at optimum levels. The Defiance area soils have somewhat higher CEC's because of their finer textures.

Crop Yields

Table 2.5 gives crop yields by farm, crop and year for the Columbus demonstration plots. These results cannot be analyzed statistically because they were not replicated, but some general observations can be made. In the corn studies, there were no differences between the fertilizer and sludge treatments. Since P and K levels were high in most of these soils (Table 2.4), the crop is probably responding to the sludge nitrogen. At the sludge application rates of 8.5 to 17.3 mt/ha used in the Columbus demonstrations, 173-353 kg available N/ha would have been applied, assuming the Columbus sludge contained 20.4 kg N/mt (Table 2.2). These levels are adequate for maximum corn growth at the yield levels obtained. On the 1981 corn study on the Shipley farm (Table 2.5), sludge was compared to a zero fertilizer check and there appears to have been a yield response to the sludge. The soybean studies showed no response to sludge compared to the zero fertilizer check. Since soybean is a legume, it does not respond to applied nitrogen, and the phosphorus and potassium levels in the soils were high enough to prevent any yield response to sludge applied P or K. The only exception was the Thomas study in 1981 (Table 2.5) where there appeared to be a yield increase with sludge. The available P level on this soil was 28 kg P/ha, slightly below the sufficiency level for soybeans.

TABLE 2.4. THE RANGES AND MEANS OF SOIL TEST RESULTS FOR FARM FIELDS RECEIVING SLUDGE IN THE STUDY

Area	No.	pH		Bray P1 Available P (kg P/ha)		Exchangeable K (kg K/ha)		Cation Exchange Capacity (meq/100g)	
		Mean	Range	Mean	Range	Mean	Range	Mean	Range
Columbus	126	6.3	4.8-7.5	57	11-310	254	121-776	16	7-40
Defiance	17	6.7	5.8-7.3	58	14-119	280	109-403	20	7-22
Medina	91	6.4	4.7-7.3	41	6-200	210	84-428	11	4-18
Springfield	25	6.5	5.6-7.5	83	34-165	265	140-390	13	9-21

TABLE 2.5. SLUDGE DEMONSTRATION PLOTS IN THE COLUMBUS STUDY (FRANKLIN, PICKAWAY AND MADISON COUNTIES)

Farm	Crop	Treatment	Yield (kg/ha)
		1979	
Hastings	Corn	Fertilizer (580 kg/ha 5-15-40)	6870
		Sludge (17.3 mt/ha)	7760
		1980	
Leatherwood	Corn	Fertilizer*	10940
		Sludge (10.5 mt/ha)	11840
McBride	Corn	Fertilizer	13590
		Sludge (9.4 mt/ha)	13350
Morris	Corn	Fertilizer	6270
		Sludge (8.5 mt/ha)	7570
		1981	
Keck	Soybeans	Check	1150
		Sludge (12.3 mt/ha)	1360
Landes	Soybeans	Check	1740
		Sludge (9.9 mt/ha)	2220
Thomas	Soybeans	Check	770
		Sludge (8.3 mt/ha)	1380
Thomas	Soybeans	Check	2020
		Sludge (7.0 mt/ha)	1980
Shipley	Soybeans	Check	2060
		Sludge (12.6 mt/ha)	1740
Shipley	Corn	Check	6990
		Sludge (10.8 mt/ha)	9210

* The recommended fertilizer application for that crop was applied unless otherwise indicated.

The Medina corn data indicate that there may have been a specific response to sludge over and above the response to fertilizer (Table 2.6). However, the amounts of available nitrogen provided by the Medina sludges are low at the rates of sludge applied, no more than 75 kg N/ha, so it does not appear that the crop is responding to crop N. Likewise, at the P soil test levels found, it is not common to observe corn yield increases of the magnitude found here. In addition, as will be seen later, there were no major differences in leaf or grain nitrogen or phosphorus that would indicate responses to either of these elements. As in the Columbus study, there was no response of soybeans to sludge. Likewise, wheat did not respond to either fertilizer or sludge additions. In contrast, both hay studies showed a response to sludge applications compared to either fertilizer or the check.

Two of the corn studies in Defiance (Table 2.7) seemed to indicate yield responses to sludge versus fertilizer applications, while a third showed little difference, and a fourth (Janicek in 1981) indicated lower yield with sludge. Oats showed little or no response to sludge versus fertilizer while sludge seemed to increase alfalfa yield compared to fertilizer or check.

The corn studies with Springfield sludge showed no effect or slightly lower yields with sludge compared to fertilizer (Table 2.8). At the lower rates of sludge application, however, only about 80 kg N/ha of available N was supplied, much less than the 150-250 kg N/ha required for optimum corn yields. The soybean study showed little effect of sludge compared to the check.

Plant Tissue Composition

Table 2.9 gives the nutrient and metal compositions of leaf and grain tissue for the Columbus sludge demonstration plots. Since these plots were not replicated, only general trends can be observed. Nitrogen was only measured in one study and the data indicate no differences between fertilizer and sludge. In all comparisons between fertilizer and sludge or check and sludge, there appeared to be very few differences in P, K or metal composition. Based on published literature values, the metal concentrations are all near normal background levels. There was some evidence for a consistent small increase in Cd concentrations in leaves but not in grain. Copper and cadmium concentrations were lower in grain than in the leaves, while there were few concentration differences between grain and leaves with the other metals.

Table 2.10 gives similar data for Medina. Many of these studies included check and fertilizer treatments and the data showed that metal uptake was as high from the fertilizer as for the sludge treatments. As in the Columbus studies, all metal concentrations were close to normal background levels. Data from the wheat crop on the Fees farm in 1979 showed somewhat higher Cd levels in the grain than in the leaf, which is opposite to what is found for corn and soybeans. Wheat is known to

TABLE 2.6. SLUDGE DEMONSTRATION PLOTS IN MEDINA COUNTY

Farm	Crop	Treatment	Yield (kg/ha)
		1978	
Knock	Legume/grass hay	Check	6500
		Fertilizer*	6400
		Sludge (3.8 mt/ha)	7800
Wagner	Corn	Check	5360
		Fertilizer	8000
		Sludge (2.2 mt/ha)	9720
		1979	
Knock	Legume/grass hay	Check	6050
		Fertilizer	4700
		Sludge (2.9 mt/ha)	7170
Fees	Wheat	Check	2940
		Fertilizer	2990
		Sludge (4.5 mt/ha)	2800
Wagner	Corn	Check	4980
		Fertilizer	7380
		Sludge (5.0 mt/ha)	8070
Wagner	Soybeans	Fertilizer	1560
		Fertilizer + sludge (1.9 mt/ha)	1700
		1980	
Wagner	Soybeans	Check	1410
		Fertilizer	2450
		Sludge (1.9 mt/ha)	1890

* The recommended fertilizer application for that crop was applied unless otherwise indicated.

TABLE 2.7. SLUDGE DEMONSTRATION PLOTS IN DEFIANCE COUNTY

Farm	Crop	Treatment	Yield (kg/ha)
		1979	
Hoshock	Corn	Fertilizer*	11,340
		Sludge (6.0 mt/ha)	10,960
Lenhart	Alfalfa	Check	6,170
		Fertilizer	6,000
		Sludge (3.0 mt/ha)	7,980
		1980	
Hoshock	Oats	Fertilizer (250 kg/ha 6-24-24)	3,040
		Sludge (11.6 mt/ha)	3,720
Janicek	Corn	Fertilizer (200 kg/ha 8-32-16; 160 kg N/ha)	13,520
		Sludge (6.1 mt/ha)	15,670
		Sludge (11.5 mt/ha)	15,330
Janicek	Corn	Fertilizer	10,660
		Sludge (6.1 mt/ha)	12,360
		Sludge (11.5 mt/ha)	11,770
		1981	
Janicek	Corn	Fertilizer	13,790
		Sludge (6.5 mt/ha)	10,690

* The recommended fertilizer application for that crop was applied unless otherwise indicated.

TABLE 2.8. SLUDGE DEMONSTRATION PLOTS IN CLARK COUNTY

Farm	Crop	Treatment	Yield (kg/ha)
		1981	
Croutwater	Soybeans	Check	1,440
		Sludge (3.9 mt/ha)	1,570
Kerns	Corn	Fertilizer*	7,470
		Sludge (4.9 mt/ha)	6,660
Greiser	Corn	Fertilizer	10,780
		Fertilizer plus sludge (9.0 mt/ha)	10,840
Thompson	Corn	Fertilizer	8,690
		Sludge (4.7 mt/ha)	7,830

* The recommended fertilizer application for that crop was applied unless otherwise indicated.

TABLE 2.9. PLANT TISSUE COMPOSITION OF CROPS GROWN WITH SEWAGE SLUDGE (COLUMBUS)

Farm	Crop	Tissue	Treatment	N	P	K	Cu	Cd	Ni	Pb	Zn
				%			μg/g				
					1979						
Hastings	Corn	Leaf	Fertilizer	2.31	--	--	23.9	0.21	0.8	2.9	35.2
			Sludge	2.38	--	--	26.6	0.24	1.3	3.4	45.6
		Grain	Fertilizer	1.10	--	--	1.1	0.02	0.8	BDL*	13.1
			Sludge	1.19	--	--	1.1	0.01	0.9	BDL	13.5
					1980						
Morris	Corn	Leaf	Fertilizer	--	0.27	2.2	7.3	0.11	0.2	1.0	21.6
			Sludge	--	0.32	2.0	8.5	0.22	BDL	0.9	44.6
		Grain	Fertilizer	--	0.31	0.34	1.1	0.05	BDL	BDL	20.0
			Sludge	--	0.34	0.32	1.3	0.05	0.3	BDL	21.9
Leatherwood	Corn	Leaf	Fertilizer	--	0.35	2.0	8.7	0.27	0.3	1.8	20.4
			Sludge	-	0.34	2.1	10.0	0.47	0.4	1.3	18.1
		Grain	Fertilizer	--	0.38	0.33	1.5	0.04	0.2	BDL	20.6
			Sludge	--	0.27	0.27	1.1	0.06	0.4	BDL	21.1
McBride	Corn	Leaf	Fertilizer	--	0.32	2.2	12.9	0.09	0.3	1.8	18.1
			Sludge	--	0.30	1.8	7.4	0.13	0.2	1.0	22.0
		Grain	Fertilizer	--	0.29	0.29	0.9	0.06	0.4	BDL	18.6
			Sludge	--	0.27	0.26	0.9	0.06	0.3	BDL	14.2
Drummond†	Soybeans	Leaf	Check	--	0.39	2.4	7.5	0.21	2.5	1.6	27.9
			Sludge	--	0.37	2.4	9.9	0.18	2.7	2.3	31.0
		Grain	Check	--	0.63	2.1	9.6	0.08	1.6	BDL	30.1
			Sludge	--	0.61	1.9	9.4	0.10	2.3	BDL	60.6

TABLE 2.9. CONTINUED

Farm	Crop	Tissue	Treatment	N	P	K	Cu	Cd	Ni	Pb	Zn
				%	%	%	μg/g	μg/g	μg/g	μg/g	μg/g
					1980						
Hastings†	Soybeans	Leaf	Check	--	0.45	2.6	6.9	0.36	3.1	2.5	43.0
			Sludge	--	0.39	2.4	8.4	0.22	4.0	3.3	42.2
Steck†	Soybeans	Leaf	Check	--	0.44	2.4	7.2	0.08	1.9	1.9	28.0
			Sludge	--	0.43	2.6	7.0	0.08	2.2	2.2	33.2
Shipley	Corn	Leaf	Check	--	0.35	2.1	7.7	0.58	0.8	8.7	34.8
			Sludge	--	0.30	1.6	6.6	0.36	0.7	11.3	28.6
		Grain	Check	--	--	--	1.4	0.13	0.4	0.9	15.8
			Sludge	--	--	--	1.3	0.14	0.6	1.0	18.5
Shipley	Soybeans	Leaf	Check	--	0.39	2.4	8.0	0.07	1.9	17.0	29.8
			Sludge	--	0.48	2.5	6.6	0.09	3.2	8.0	34.0
Lander	Soybeans	Leaf	Check	--	0.45	2.3	6.4	0.17	3.2	30.3	28.9
			Sludge	--	0.45	2.5	7.0	0.25	2.6	15.1	31.9
Thomas	Soybeans	Grain	Check	--	0.53	1.6	7.7	0.06	3.0	2.1	27.9
			Sludge	--	0.51	1.7	7.8	0.10	2.5	1.9	30.6
Keck	Soybeans	Grain	Check	--	0.50	1.9	7.6	0.10	6.1	1.7	35.7
			Sludge	--	0.52	1.6	7.9	0.10	5.3	1.8	39.6

* Below detection limit.
† Yields were not taken on these plots.

TABLE 2.10. PLANT TISSUE COMPOSITION OF CROPS GROWN WITH SEWAGE SLUDGE (MEDINA)

Farm	Crop	Tissue	Treatment	N	P	K	Cd	Cu	Ni	Pb	Zn
				----------	%	----------	————	————	µg/g	————	————
				1978							
Knock	Legume/grass hay	Whole plant	Check	3.1	0.28	0.38	0.21	11.0	0.8	5.0	30
			Fertilizer	2.8	0.27	0.37	0.56	11.0	0.7	2.8	29
			Sludge	2.8	0.27	0.39	0.16	10.0	0.7	2.6	29
Wagner	Corn	Leaf	Check	2.6	0.20	0.37	0.20	13.0	0.3	4.0	23
			Fertilizer	2.4	0.20	0.40	0.30	14.0	0.3	2.0	20
			Sludge	2.5	0.19	0.41	0.20	12.0	0.3	3.0	20
		Grain	Check	1.3	0.26	0.19	0.04	3.0	0.3	0.2	20
			Fertilizer	1.5	0.23	0.19	0.02	3.0	0.3	0.2	19
			Sludge	1.6	0.32	0.17	0.06	3.0	0.3	0.4	22
				1979							
Knock	Legume/grass hay	Whole plant	Check	1.8	0.10	0.73	0.03	8.0	0.3	1.7	17
			Fertilizer	1.7	0.13	0.76	0.07	8.0	0.6	1.7	19
			Sludge	1.4	0.10	0.88	0.02	7.0	0.2	1.3	17
Fees	Wheat	Leaf	Check	2.9	0.35	1.0	0.02	--	1.5	2.9	35
			Fertilizer	2.4	0.26	1.0	0.05	--	2.6	5.6	41
			Sludge	2.5	0.32	0.9	0.09	25.0	1.7	4.0	76
		Grain	Check	2.1	0.20	0.6	0.18	8.5	0.5	0.8	42
			Fertilizer	1.9	0.20	0.4	0.13	10.3	0.6	0.8	47
			Sludge	1.8	0.20	0.5	0.07	9.0	0.4	0.9	37
		Straw	Check	0.37	0.04	0.06	0.39	9.0	0.04	2.2	20
			Fertilizer	0.31	0.04	0.05	0.22	5.0	0.30	2.2	13
			Sludge	0.27	0.04	0.05	0.11	--	0.20	1.8	10

TABLE 2.10. CONTINUED

Farm	Crop	Tissue	Treatment	N	P	K	Cd	Cu	Ni	Pb	Zn
				%			µg/g				
Wagner	Corn	Leaf	Check	--	--	--	--	--	--	--	--
			Fertilizer	1.9	--	--	0.15	13.0	1.0	2.1	14
			Sludge	2.2	--	--	0.15	15.0	0.9	2.5	18
		Grain	Check	1.0	--	--	0.04	1.2	0.5	BDL*	14
			Fertilizer	1.0	--	--	0.04	0.9	0.5	0.1	13
			Sludge	1.2	--	--	0.04	1.3	0.4	BDL	15
Wagner	Soybeans	Leaf	Fertilizer	4.7	--	--	0.14	11.0	3.3	1.5	30
			Fertilizer plus sludge	5.1	--	--	0.17	11.0	3.7	1.8	27
		Grain	Fertilizer	6.8	--	--	0.11	12.0	2.2	BDL	34
			Fertilizer plus sludge	7.2	--	--	0.08	11.0	2.7	BDL	37
				1980							
Wagner	Soybeans	Grain	Check	6.8	0.61	1.96	0.07	9.0	1.2	BDL	26
			Fertilizer	7.2	0.64	1.91	0.08	10.0	1.2	BDL	28
			Sludge	6.9	0.62	1.87	0.09	10.0	1.5	BDL	28

* Below detection limit.

concentrate cadmium somewhat in the grain, but the grain concentrations found here are still very low.

The Defiance data (Table 2.11) gives results similar to those reported for the other areas except for the two studies on the Janicek farm in 1980 and 1981 which indicated high leaf cadmium concentrations and one high Zn concentration. The grain levels, however, were low. This finding could have been due to the particular corn hybrid used since there are significant differences among corn hybrids (CAST, 1980) in metal uptake, or to contamination of the leaf tissue by dust containing sludge. Even these higher values, however, are much lower than those found on soils receiving large quantities of sludges high in metals (CAST, 1980).

The Springfield data (Table 2.12) shows results for corn and soybeans that are very similar to those found for the other areas.

Table 2.13 summarizes the corn and soybean studies for which both leaf and grain data were available for cadmium and zinc. The data show small increases in leaf Cd only, with no increases in leaf Zn or grain Cd and Zn. These results clearly demonstrate the low potential for metal accumulation at low annual rates of sludge application with sludges containing as high as 80 and 8600 μg/g sludge of Cd and Zn, respectively (Table 2.1). This is particularly true of corn and soybean grain where concentrations are lower than in the leaf.

Soil Analysis Before and After Sludge Application

All soils receiving sludge in the project were sampled prior to sludge application and analyzed for total metals in addition to the routine soil fertility tests previously discussed. At the end of the study, as many of these sites as possible were resampled; the original samples and the post-application samples were analyzed together (to eliminate operational errors) for Bray P1 phosphate and Cu, Cd, Ni, Pb, Zn and Cr. The results are given in Tables 2.14-2.16 by County. The most consistent changes were in Bray P1 and cadmium. Changes in the other metals were very low and sampling errors in many cases made it impossible to detect differences. There was a substantial increase in Bray P1 in most cases, reflecting the large amounts of P added with sludges even at agronomic rates of application. There was also a consistent increase in Cd in soil with sludge application even though Cd levels are low compared to other metals. This is primarily because the Cd concentrations in the sludges used in the study were higher compared to background soil Cd levels than similar relationships for the other metals. The variability in soil sampling and the small increases in metal levels above background concentrations when agronomic rates of sludge are applied make it difficult to use soil analysis as a means of monitoring metal additions to soil with sludge. It is more accurate to monitor metal additions by calculation from sludge application rates and sludge analyses. Periodic metal analysis is only warranted when sludge is applied to the same site for many years.

TABLE 2.11. PLANT TISSUE COMPOSITION OF CROPS GROWN WITH SEWAGE SLUDGE (DEFIANCE)

Farm	Crop	Tissue	Treatment	N	P	K	Cd	Cu	Ni	Pb	Zn
				----- % -----			µg/g				
				1979							
Hoshock	Corn	Grain	Fertilizer	1.5	--	--	0.09	2.1	0.4	BDL*	17.0
			Sludge	1.3	--	--	0.10	2.1	0.6	BDL	18.2
Lenhart	Alfalfa	Whole plant	Check	--	--	--	0.24	9.8	1.0	BDL	20.0
			Fertilizer	--	--	--	0.04	15.8	1.2	BDL	24.1
			Sludge	--	--	--	0.08	9.4	1.5	BDL	20.4
				1980							
Hoshock	Oats	Grain	Fertilizer	--	0.43	0.37	0.10	3.7	1.0	BDL	26.0
			Sludge	--	0.42	0.41	0.06	3.5	1.0	BDL	22.4
Janicek	Corn	Leaves	Fertilizer	--	0.40	1.7	0.75	9.9	BDL	8.5	23.7
			Sludge (6.1 mt/ha)	--	0.37	1.6	0.98	9.0	BDL	6.5	19.9
			Sludge (11.5 mt/ha)	-- --	0.40	2.2	1.44	8.6	BDL	12.1	74.9
		Grain	Fertilizer	--	0.36	0.32	0.11	1.6	0.1	BDL	25.9
			Sludge (6.1 mt/ha)	--	0.24	0.24	0.11	1.9	0.2	BDL	19.2
			Sludge (11.5 mt/ha)	--	0.37	0.33	0.08	1.9	BDL	BDL	19.4
				1981							
Janicek	Corn	Leaf	Fertilizer	--	0.33	1.7	0.42	8.3	0.9	12.8	25.6
			Sludge	--	0.30	1.7	0.84	11.3	0.9	19.6	23.6
		Grain	Fertilizer	--	--	--	0.09	1.5	0.3	BDL	14.2
			Sludge	--	--	--	0.08	1.3	0.4	0.8	13.6

* Below detection limit.

TABLE 2.12. PLANT TISSUE COMPOSITION OF CROPS GROWN WITH SEWAGE SLUDGE (SPRINGFIELD)

Farm	Crop	Tissue	Treatment	N	P	K	Cd	Cu	Ni	Pb	Zn
				%			µg/g				
				1981							
Croutwater	Soybeans	Leaf	Check	--	0.29	1.6	0.18	6.6	1.0	5.7	24.1
			Sludge	--	0.37	2.2	0.32	7.8	1.7	3.9	26.8
		Grain	Check	6.4	0.61	1.9	0.08	7.5	2.0	1.7	27.1
			Sludge	6.2	0.66	2.0	0.06	6.9	2.2	1.9	31.5
Kerns	Corn	Leaf	Fertilizer	--	0.30	1.8	0.11	9.7	1.3	9.8	25.1
			Sludge	--	0.36	1.8	0.19	9.9	0.9	6.0	33.9
		Grain	Fertilizer	1.6	--	--	0.03	1.4	0.4	1.0	13.4
			Sludge	1.7	--	--	0.04	1.0	0.4	1.0	11.8
Greiser	Corn	Leaf	Fertilizer	--	0.33	2.3	0.11	23.0	1.1	4.0	23.0
			Fertilizer plus sludge	--	0.26	2.1	0.09	15.0	1.1	4.0	27.0
		Grain	Fertilizer	1.4	--	--	0.03	0.9	0.4	0.9	10.3
			Fertilizer plus sludge	1.3	--	--	0.02	1.1	0.4	0.8	12.5
Thompson	Corn	Leaf	Fertilizer	--	0.30	2.5	0.64	12.0	0.9	8.1	36.4
			Sludge	--	0.30	1.8	0.40	11.6	0.8	15.1	13.3
		Grain	Fertilizer	1.6	--	--	0.04	1.0	0.4	0.6	13.1
			Sludge	1.6	--	--	0.08	1.0	0.5	BDL *	13.3

* Below detection limit.

TABLE 2.13. AVERAGE RATES OF APPLICATION OF CADMIUM AND ZINC APPLIED WITH SLUDGE IN THE DEMONSTRATION PLOTS AND THEIR EFFECTS ON CD AND ZN UPTAKE BY CORN AND SOYBEANS

Crop	Number of Observations	Sludge Rate mt/ha	Metal Added		Treatment	Leaf		Grain	
			Cd	Zn		Cd	Zn	Cd	Zn
			kg/ha			μg/g			
Corn	12	8.2	0.43	37.3	Control*	0.29	25.0	0.05	16.8
					Sludge	0.41	30.5	0.06	16.9
Soybeans	3	2.6	0.17	12.4	Control*	0.16	27.1	0.09	29.0
					Sludge	0.25	26.9	0.08	32.2

* Either a zero fertilizer check or a fertilizer control.

TABLE 2.14. BRAY P1 AND TOTAL METAL CONTENTS OF FARM FIELDS BEFORE AND AFTER SLUDGE APPLICATION (COLUMBUS)

Site No.	Bray P1		Cu		Cd		Ni		Pb		Zn		Cr	
	B*	A*	B	A	B	A	B	A	B	A	B	A	B	A
	μg/g													
1	32.3	109.4	10.0	13.2	BDL	0.26	9.8	14.0	9.2	13.7	43.3	69.8	5.6	9.8
2	35.5	68.3	17.4	21.6	0.19	0.36	19.5	20.7	14.2	19.6	68.4	84.2	10.5	11.8
3	67.4	23.1	13.7	13.1	0.19	0.17	14.3	15.1	10.7	11.6	55.9	53.7	6.8	8.0
4	421.4	345.9	15.0	31.1	0.33	2.27	9.4	23.2	23.1	43.6	141.9	378.6	9.9	25.6
5	37.8	115.4	10.5	14.9	BDL	0.52	8.8	13.4	11.8	19.3	47.8	93.1	8.2	11.2
6	32.8	85.4	10.5	14.3	0.25	0.45	16.9	12.2	11.5	10.5	54.9	61.5	6.0	7.9
7	49.2	300.9	14.2	16.5	0.14	0.51	16.8	11.7	16.4	17.3	55.2	85.4	7.4	12.2
8	21.0	56.8	16.5	20.6	0.14	0.25	17.4	16.9	11.9	13.1	62.6	76.2	11.6	11.0
9	10.2	24.0	12.2	16.7	0.12	0.30	13.4	13.2	12.0	16.5	44.2	59.2	7.3	8.8
10	54.9	102.9	14.7	11.0	0.22	0.28	14.0	12.9	11.9	12.0	56.8	56.3	6.2	6.0
11	20.3	47.1	15.0	10.7	0.14	0.20	15.6	9.0	14.7	9.1	57.6	40.4	8.8	5.5
12	39.2	139.6	14.0	13.6	0.15	0.44	17.2	11.3	13.3	12.5	62.7	59.7	10.6	8.8
13	19.2	19.6	15.1	11.8	0.11	0.17	15.7	11.9	13.2	8.3	52.2	55.6	10.5	8.8
14	33.2	121.1	14.0	16.6	0.21	0.57	12.8	14.7	11.9	13.7	50.0	68.2	9.7	10.7
15	43.8	57.5	12.3	12.0	0.15	0.20	16.2	12.1	15.2	11.3	56.3	56.2	8.3	6.2
16	11.1	74.3	18.0	18.7	0.20	0.34	16.4	14.0	15.2	13.8	65.7	53.3	10.7	9.7
17	16.6	28.2	18.5	10.0	0.18	0.21	16.9	8.7	14.9	9.8	63.3	38.5	10.9	5.2
18	188.5	39.5	14.3	15.6	0.28	0.25	14.1	16.5	15.8	8.9	65.6	63.2	7.0	5.3
19	18.5	38.8	15.4	11.6	0.22	0.23	16.6	11.2	14.5	10.2	57.6	54.6	7.9	9.2
20	8.3	24.9	15.1	20.5	0.16	0.34	14.8	18.1	13.4	16.1	56.1	69.1	10.2	11.3
21	32.3	66.0	17.9	15.3	0.21	0.32	29.7	13.7	13.1	14.1	69.9	71.4	10.9	14.1
22	25.6	81.9	15.5	17.2	0.15	0.38	14.7	14.9	14.5	18.0	57.6	75.8	9.7	12.8
23	30.0	30.0	15.6	12.6	0.14	0.16	16.0	9.6	15.1	14.6	78.0	52.2	8.8	9.0
24	6.9	16.6	13.4	11.3	BDL	0.11	13.5	10.1	11.7	11.8	45.7	42.4	7.5	7.5
25	109.8	145.6	13.5	16.9	0.14	0.54	13.6	15.4	13.2	18.3	57.1	143.3	9.7	11.8
Mean	52.1	88.4	14.2	15.4	0.18	0.41	15.0	14.9	13.5	14.9	59.8	78.2	8.7	9.8
C.V.(%)	158.3	89.0	17.2	29.1	31.4	99.4	27.1	25.4	19.8	46.9	31.0	81.4	22.4	40.6

* B = Before sludge application; A = After sludge application.

TABLE 2.15. BRAY P1 AND TOTAL METAL CONTENTS OF FARM FIELDS BEFORE AND AFTER SLUDGE APPLICATION (MEDINA)

Site No.	Bray P1		Cu		Cd		Ni		Pb		Zn		Cr	
	B*	A*	B	A	B	A	B	A	B	A	B	A	B	A
	µg/g													
1	31.4	37.2	24.1	14.1	0.24	0.25	12.8	14.2	12.3	11.5	48.1	55.3	6.5	8.9
2	8.8	52.6	10.5	11.9	0.18	0.16	11.6	12.3	10.5	10.6	44.1	46.2	5.9	6.2
3	12.9	223.4	10.0	11.4	0.09	0.14	10.8	11.2	10.5	11.8	40.8	44.5	6.0	5.9
4	14.3	24.5	10.4	8.2	0.18	0.16	9.8	9.2	12.0	10.6	52.0	49.0	6.4	5.9
5	41.5	201.7	10.1	10.3	0.22	0.18	9.7	8.7	9.0	9.0	41.6	37.6	5.3	5.3
6	8.8	50.3	10.1	11.5	0.19	0.23	10.7	11.3	11.5	12.8	47.8	56.6	6.4	7.0
Mean	19.6	98.3	12.5	11.2	0.18	0.19	10.9	11.1	11.0	11.2	45.7	48.2	6.0	6.6
CV(%)	69.4	90.9	45.2	17.3	28.2	23.4	10.7	11.8	11.2	11.8	9.4	14.7	9.3	19.5

* B = Before sludge application; A = After sludge application.

TABLE 2.16. BRAY P1 AND TOTAL METAL CONTENTS OF FARM FIELDS BEFORE AND AFTER SLUDGE APPLICATION (DEFIANCE AND SPRINGFIELD)

Site No.	Bray P1		Cu		Cd		Ni		Pb		Zn		Cr	
	B*	A*	B	A	B	A	B	A	B	A	B	A	B	A
	μg/g													
Defiance														
1	57.2	16.2	16.7	18.5	0.27	0.29	17.8	19.8	11.5	11.1	52.5	62.8	13.7	16.5
2	35.1	62.8	9.9	16.7	0.22	0.27	10.5	17.8	6.1	11.5	31.9	52.5	8.7	13.7
3	42.9	201.7	14.2	15.1	0.28	0.33	14.6	9.8	8.9	10.7	47.8	41.6	11.4	9.7
Mean	45.1	73.4	15.2	13.2	0.28	0.26	14.2	12.6	9.3	9.2	43.3	44.5	11.2	11.0
CV(%)	24.9	120.6	8.9	33.3	2.1	23.3	26.4	38.0	21.8	24.6	27.9	29.3	23.3	33.3
Springfield														
1	46.2	82.6	6.1	5.7	0.10	0.11	5.3	5.5	5.8	6.0	26.1	27.8	6.7	7.1
2	29.8	42.0	7.3	7.8	0.18	0.17	6.2	6.5	7.2	7.1	41.2	36.7	7.1	6.7
3	26.8	116.1	13.3	12.6	0.17	0.18	13.4	11.9	12.6	12.9	47.0	53.2	9.8	11.7
4	76.6	95.5	9.8	9.4	0.16	0.16	9.7	7.7	11.4	11.6	41.3	41.6	10.9	10.2
5	38.8	53.5	10.3	11.2	0.10	0.16	11.6	11.9	7.9	9.8	35.9	43.8	8.7	9.5
6	73.8	223.1	12.0	12.5	0.13	0.32	13.2	11.7	10.1	15.0	45.6	69.9	9.8	15.4
7	33.7	87.0	11.2	13.5	0.12	0.17	11.6	12.1	8.6	12.2	39.0	55.4	9.5	11.0
Mean	46.5	100.0	10.0	10.4	0.14	0.18	10.1	9.6	9.1	10.7	39.4	47.2	8.9	10.2
CV(%)	44.3	59.8	25.5	27.6	24.1	35.9	32.0	30.4	26.5	30.2	17.7	29.1	17.2	28.9

* B = Before sludge application; A = After sludge application.

A COMPUTER PROGRAM FOR SLUDGE APPLICATION TO AGRICULTURAL LAND

As an aid in determining nutrient and metal additions to cropland and supplemental nutrient requirements, a computer program was written for use on a microcomputer. The inputs to the program (Figure 2.21) include soil analysis, sludge analysis and specification of previous and next crop and yield goal. The program uses current fertilizer recommendations of the Ohio State University Cooperative Extension Service (Agronomy Guide, 1983) for corn, soybeans, wheat, small grains (oats rye, barley) and hay (grasses or legumes) to determine nutrient requirements, and current fertilizer prices to calculate value of sludge nutrients. The following steps are followed in the program:

1. The crop to be grown and the farmer's yield goal is determined and the previous crop is identified.

2. A standard fertility soil test is run. This includes: pH; lime requirement (to pH 6.5); Bray P1 available P; exchangeable K, Mg, Ca; cation exchange capacity.

3. Items 1 and 2 are then used to determine the nitrogen, phosphorus and potassium requirements of the crop.

4. The sludge is analyzed for: pH, solids, NH_3-N, organic-N, P, K, Cd, Cu, Ni, Pb, Zn.

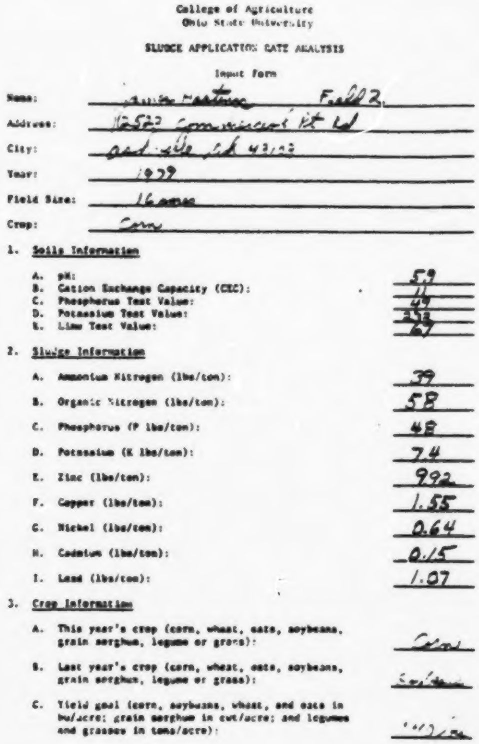
5. The sludge analysis data is used to determine the N, P and K nutrients and the five metals supplied per ton of sludge.

6. It is assumed that all of the P and K in sludge is equivalent to fertilizer and all of the NH_3-N is available. It is also assumed that 30% of the organic-N is available in the year it is applied. In addition, the program assumes that none of the remaining organic-N (70%) is available in subsequent years. Finally, the program assumes that P and K not utilized by the crop is as available as fertilizer P and K in subsequent years. These assumptions are not entirely valid and can be improved with further research. For example, research indicates that 5-30% of the NH_3-N can volatilize if the sludge is not immediately incorporated. Also, sludge phosphorus is probably not completely available (Pastene and Corey, 1978), although data on sludge P availability is limited.

7. The program calculates and prints out (Figure 2.22) the N, P and K supplied by 1-6 tons/acre (2.2-13.2 mt/ha) of sludge and compares these values with the NPK requirements of the crop. The farmer can then choose to apply enough sludge to apply one or more of these three elements, and he can determine supplemental N, P and K he may need.

8. The program then calculates the dollar value of 1-6 tons/acre sludge based on the crop utilization of available nutrients. It

(This is the best copy available; we regret that portions are undecipherable.)



College of Agriculture
Ohio State University

SLUDGE APPLICATION RATE ANALYSIS

Input Form

Name: [illegible] Field 2
Address: 12523 Commercial Pt Rd
City: Ashville Oh 43103
Year: 1979
Field Size: 16 acres
Crop: Corn

1. Soils Information

A. pH:	5.9
B. Cation Exchange Capacity (CEC):	11
C. Phosphorus Test Value:	49
D. Potassium Test Value:	252
E. Lime Test Value:	67

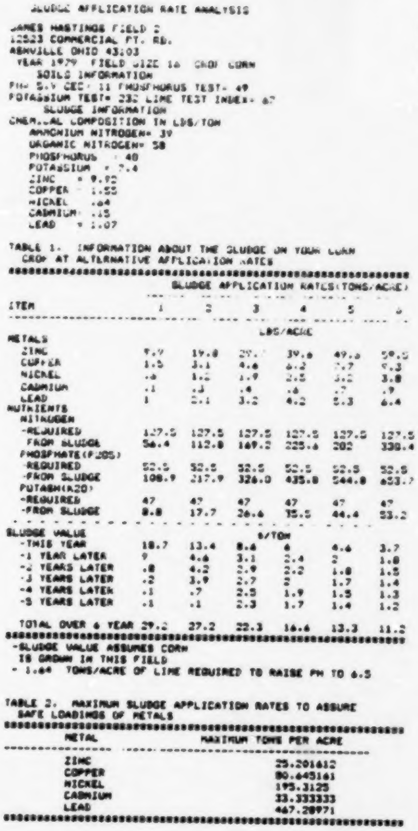
2. Sludge Information

A. Ammonium Nitrogen (lbs/ton):	39
B. Organic Nitrogen (lbs/ton):	58
C. Phosphorus (P lbs/ton):	48
D. Potassium (K lbs/ton):	74
E. Zinc (lbs/ton):	992
F. Copper (lbs/ton):	1.55
G. Nickel (lbs/ton):	0.64
H. Cadmium (lbs/ton):	0.15
I. Lead (lbs/ton):	1.07

3. Crop Information

A. This year's crop (corn, wheat, oats, soybeans, grain sorghum, legume or grass):	Corn
B. Last year's crop (corn, wheat, oats, soybeans, grain sorghum, legume or grass):	Soybeans
C. Yield goal (corn, soybeans, wheat, and oats in bu/acre; grain sorghum in cwt/acre; and legumes and grasses in tons/acre):	[illegible]

Figure 2.21. Data input form for sludge analysis computer program.



```
    SLUDGE APPLICATION RATE ANALYSIS

JAMES HASTINGS FIELD 2
12523 COMMERCIAL PT. RD.
ASHVILLE OHIO 43103
 YEAR 1979  FIELD SIZE 16  CROP CORN
    SOILS INFORMATION
PH= 5.9 CEC= 11 PHOSPHORUS TEST= 49
POTASSIUM TEST= 232 LIME TEST INDEX= 67
     SLUDGE INFORMATION
CHEMICAL COMPOSITION IN LBS/TON
   AMMONIUM NITROGEN= 39
   ORGANIC NITROGEN= 58
   PHOSPHORUS  = 40
   POTASSIUM  = 7.4
   ZINC    = 9.92
   COPPER  = 1.55
   NICKEL   .64
   CADMIUM= .15
   LEAD    = 1.07

TABLE 1.  INFORMATION ABOUT THE SLUDGE ON YOUR CORN
  CROP AT ALTERNATIVE APPLICATION RATES
#################################################################
                          SLUDGE APPLICATION RATES(TONS/ACRE)
                         ----------------------------------------
ITEM                         1      2      3      4      5      6
-----------------------------------------------------------------
                                           LBS/ACRE
METALS
   ZINC                  9.9    19.8   29.7   39.6   49.5   59.5
   COPPER                1.5    3.1    4.6    6.2    7.7    9.3
   NICKEL                .6     1.2    1.9    2.5    3.2    3.8
   CADMIUM               .1     .3     .4     .6     .7     .9
   LEAD                  1      2.1    3.2    4.2    5.3    6.4
NUTRIENTS
   NITROGEN
   -REQUIRED             127.5  127.5  127.5  127.5  127.5  127.5
    FROM SLUDGE          56.4   112.8  169.2  225.6  282    338.4
   PHOSPHATE(P2O5)
   -REQUIRED             52.5   52.5   52.5   52.5   52.5   52.5
   -FROM SLUDGE          108.9  217.9  326.8  435.8  544.8  653.7
   POTASH(K2O)
   -REQUIRED             47     47     47     47     47     47
   -FROM SLUDGE          8.8    17.7   26.6   35.5   44.4   53.2
- - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -
SLUDGE VALUE                               $/TON
   -THIS YEAR            18.7   13.4   8.6    6      4.6    3.7
   -1 YEAR LATER         9      4.6    3.1    2.4    2      1.8
   -2 YEARS LATER        .8     4.2    2.9    2.2    1.8    1.5
   -3 YEARS LATER        .2     3.9    2.7    2      1.7    1.4
   -4 YEARS LATER        .1     .7     2.5    1.9    1.5    1.3
   -5 YEARS LATER        .1     .1     2.3    1.7    1.4    1.2

   TOTAL OVER 6 YEAR 29.2   27.2   22.3   16.6   13.3   11.2
#################################################################
 -SLUDGE VALUE ASSUMES CORN
  IS GROWN IN THIS FIELD
 - 1.64  TONS/ACRE OF LIME REQUIRED TO RAISE PH TO 6.5

TABLE 2.  MAXIMUM SLUDGE APPLICATION RATES TO ASSURE
  SAFE LOADINGS OF METALS
#################################################################
        METAL                 MAXIMUM TONS PER ACRE
---------------   ---------------------------------------
        ZINC                              25.201612
        COPPER                            80.645161
        NICKEL                            195.3125
        CADMIUM                           33.333333
        LEAD                              467.28971
#################################################################
```

(This is the best copy available; we regret that portions are undecipherable.)

Figure 2.22. Output of sludge analysis computer program.

does not place any value on nitrogen not utilized by the crop in the year applied, but it does place a value on P and K carried over for a period of five years after the year of application. Current fertilizer costs are used to calculate sludge value.

9. The metals applied with sludge at rates between 1 and 6 tons/acre (2.2 and 13.2 mt/ha) are calculated and displayed (Figure 2.22). This data is also used to calculate the maximum cumulative amount of that sludge that can be applied to that soil based on Ohio's guidelines for cumulative metal additions (Ohio Sludge Guide, 1982).

This computer program was extremely useful in the project for several reasons: (1) it was an effective tool in demonstrating to farmers the contents and economic value of sludge nutrients, as well as the contents and sludge-loading limitations of heavy metals in sewage sludge; (2) it was an effective means of documenting the land application process.

This computer program has since been modified and adopted by the REAL laboratory of the Ohio Agricultural Research and Development Center, Wooster, Ohio, and is used to give recommendations on sludge use for crops in Ohio based on submitted soil and sludge samples.

FARM SCIENCE REVIEW FIELD DEMONSTRATION

Introduction

In 1978, a replicated field research study was initiated at the Ohio State University Farm Science Review at Don Scott Airport, Columbus. The objective of the study was to collect more detailed data than could be obtained from the farmer demonstrations on the use of sewage sludge at agronomic rates of application. Also, because of its location at the Farm Science Review, site of the annual state-wide farm show, this experiment was effectively used as a demonstration tool for the state-wide farmer audience. Signs detailing the experimental treatments were posted each year, and the annual yield results were published in the Agronomy Department bulletin prepared for each Review. In addition, members of the project staff were available at the plots during the Review to answer questions.

Methods

Centrifuged Columbus Jackson Pike anaerobically digested sewage sludge was used for the study. The required quantity was hauled to the site and stockpiled no more than two days prior to spreading. A small front-end loader was used to transfer sludge to a commercial side-throw flail manure spreader (Figure 2.23). The rate of application was measured by running the spreader at a designated speed and rpm and then collecting and weighing sludge on sheets of paper 45 cm x 45 cm. This gave a rate of 6.5 mt/ha dry sludge in 1978, 1979, 1981 and 1982 and 5.5 mt/ha in 1980. A double rate was also applied (11 and 13 mt/ha)

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Figure 2.23. Side-throw manure spreader used to spread Columbus cake sludge at OSU Farm Science Review Research plots.

each year by spreading on the same plot twice. The sludge was incorporated by moldboard plowing and disking within 24 hours of spreading and the crop was planted within two days after disking. In the fall of 1978, sludge was applied at the half rate prior to planting wheat but no sludge was applied to the full rate plots for wheat. The sludge treatments were repeated on the plots and agricultural lime was added in the fall of 1978, after the first crop, to raise soil pH to 6.5 Because of variability in soil pH among the plots and the small difference between unlimed and limed pH ($\sim$ 6.0 vs 6.5), this treatment gave no differences between any of the dependent variables studied. These additional sludge plots were, therefore, treated as replicates of the sludge treatments. All treatments were replicated twice, with one replicate cropped with corn each year and the other in a corn-wheat-corn-soybean-corn rotation. In addition, all treatment combinations were replicated in three blocks, with treatments randomized within each block. Each plot was 9.1 m wide and 30.5 m long except the fertilizer and control plots, each of which was 9.1 m wide and 15.25 m long and placed next to each other to give the same dimensions as the other plots. A 10.7 m buffer area was left between each block as a turn path for the spreader.

The fertilizer treatment for corn was 220 kg N/ha as NH_4NO_3 and 40 kg P/ha as monocalcium phosphate, applied by hand just before plowing. A blanket application of 65-200 kg K/ha was applied to the entire experiment each spring depending on the potassium soil test so as to eliminate potassium as a variable in the study. About 40 kg P/ha was applied in the spring to the fertilizer plots prior to planting soybeans but no nitrogen was added. About 50 kg N/ha and 50 kg P/ha were added to the fertilizer plots just prior to planting wheat.

Soil samples were taken from each plot in the spring of 1978 before the start of the experiment and, thereafter, in the fall after crop harvest. The soils were analyzed for: pH; Bray P1 available P; DTPA extractable and total Cd, Cu, Ni, Pb and Zn. Plant leaves were sampled at tasseling (corn), flower initiation (soybeans) or heading (wheat) and analyzed for Cd, Cu, Ni, Pb and Zn. Crop yields were measured by hand harvesting 15 m of row. Grain was analyzed for Cd, Cu, Ni, Pb and Zn. Analytical procedures are those which were previously discussed.

Results

Sludge Analysis--

The analyses of Columbus sludge used each year are given in Table 2.17. These were used to calculate the annual additions of N, P, K and Cd, and the cumulative addition of Cd, Cu, Ni, Pb and Zn (Table 2.18). The nitrogen supplied by the half rate of sludge was probably low for corn, as was the full rate in 1979. However, this soil (Crosby silt loam with some Kokomo loam) has a fairly high organic matter content (3-5%) and N mineralization from the soil is probably substantial. Phosphorus supplied by sludge was high at all rates, but potassium supply was only half of what a corn crop would normally require on this soil. However, potash was applied uniformly to the

TABLE 2.17. ANALYSIS OF THE COLUMBUS JACKSON PIKE ANAEROBICALLY DIGESTED SEWAGE SLUDGE USED IN THE STUDY (1978-1982)

	pH	Solids %	NH_3-N	Organic N	Avail. N*	P	K.	Cd	Cu	Ni	Pb	Zn	Cr
			kg/mt dry sludge										
1978	7.3	--†	6.0‡	29.2	14.8	27.5	4.9	0.059	0.71	0.33	0.63	5.10	--
1979	7.3	--†	6.0	19.3	11.8	26.8	4.2	0.143	0.80	0.29	0.30	5.66	0.63
1980	7.8	19.7	6.4	32.9	15.6	25.2	4.1	0.061	0.73	0.51	0.87	5.63	0.99
1981	8.1	16.8	7.9	42.8	20.0	25.1	4.1	0.095	0.72	0.33	0.61	4.62	0.59
1982	7.6	19.5	4.4	29.8	12.9	25.5	4.4	0.061	0.69	0.23	0.48	3.27	0.51

* Assumes 30% of the organic-N is available and that 10% of the NH_3-N is lost by volatilization.

† The solids on the centrifuged sludge was not analyzed in this period.

‡ Estimated from the mean of monthly analyses for 1978-1982.

TABLE 2.18. ANNUAL RATES OF APPLICATION OF N, P, K AND CD, AND CUMULATIVE APPLICATIONS OF CD, CU, NI, PB AND ZN (1978-1982)

Year	Sludge Rate (mt/ha)	Annual Loading (kg/ha/yr)				Cumulative Loading (kg/ha)				
		Avail. N*	P	K	Cd	Cd	Cu	Ni	Pb	Zn
1978	6.5	96	179	32	0.38	0.38	4.6	2.1	4.1	33.2
	13.0	192	358	64	0.76	0.76	9.2	4.2	8.2	66.4
1979	6.5	77	174	27	0.93	1.31	9.8	4.0	6.1	70.0
	13.0	154	348	54	1.86	2.62	19.6	8.0	12.2	140.0
1980	5.5	86	139	23	0.34	1.65	13.8	6.8	10.9	101.0
	11.0	172	278	46	0.68	3.30	27.6	13.6	21.8	202.0
1981	6.5	130	163	27	0.62	2.27	18.5	8.9	14.9	131.0
	13.0	260	326	54	1.24	4.54	37.0	17.8	29.8	262.0
1982	6.5	84	166	29	0.40	2.67	23.0	10.4	18.0	152.3
	13.0	168	332	58	0.80	5.34	46.0	20.8	36.0	304.6

* Assumes 30% of the organic-N is available and that 10% of the NH_3-N is lost by volatilization.

experiment area each year, so potassium supply would not be limiting. Annual Cd application rate was below the 2.0 kg/ha/yr allowed by EPA (EPA, 1979) in all years, although the 1.86 kg/ha applied in 1979 approached this value. Cumulative amounts of the five metals studied were well within the levels allowed by Ohio EPA (Ohio Sludge Guide, 1982) for a soil with a CEC between 5 and 15 meq/100 g.

Yields--

Crop yields are given in Table 2.19. There was a response of corn to fertilizer or sludge in all years except the first. The area used for this experiment had been used for corn previously, and there may have been carry over of residual nitrogen which affected corn response to sludge or fertilizer N in 1978. This is suggested by the high check plot yield (9,030 kg/ha) in that year compared to 3500-7000 kg/ha in following years. There were no differences between the fertilizer and full-rate sludge treatments, while the half rate of sludge tended to be lower than the full rate or the fertilizer treatments except in 1978.

There was a significant response of wheat to both rates of sludge addition. This was a nitrogen response, and the fertilizer treatment did not give higher yields because the wheat on this treatment matured more rapidly and lodged before it could be harvested. There was also some shattering of seed heads before and during harvesting which contributed to yield loss. There was slower maturity and no lodging on the sludge plots.

There were no yield differences on the soybean plots. This was expected since soybeans fix their own nitrogen and phosphorus soil tests were high enough to preclude a response to P.

Leaf and Grain Analysis--

The nutrient (NPK) and metal contents of corn leaves and grain are given in Table 2.20. Different hybrids were used each year, so comparisons should not be made from one year's results to another. However, the effect of repeated sludge applications can be made by comparing the change in the elemental composition of corn tissue from the sludge-treated plots with concentrations from the control and fertilizer plots.

Nitrogen in the leaf was only evaluated in 1978, and results show that the half-rate of sludge had significantly lower N concentration than the fertilizer treatment, while the full rate was the same as the fertilizer treatment. Table 2.18 indicates that the half rate in 1978 only supplied 96 kg N/ha, about half the normal N needs of corn. There were no treatment differences in grain N in 1978 and 1979, but grain N was significantly lower on the sludge treatments than the fertilizer treatment in 1981.

There were no differences in leaf P in any year except 1982 where leaf P on the control plots were lower than the fertilizer or sludge treatments. Grain P generally showed few consistent differences among treatments. The lack of response of tissue P to the treatments is not

TABLE 2.19. CROP YIELDS ON THE SLUDGE PLOTS (1978-1982)

Treatment	Corn 1978	Corn 1979	Corn 1980	Corn 1981	Corn 1982	Wheat† 1979	Soybeans 1981
	kg/ha						
Control	9,030 b*	4,700 b	7,090 c	3,510 c	6,020 b	1950 b	2820 a
Fertilizer‡	10,040 ab	7,840 a	9,720 a	6,840 a	8,090 a	2020 b	2820 a
Full Rate‡	9,720 ab	7,840 a	9,910 a	6,520 a	6,960 ab	3900 a	2690 a
Half Rate	10,410 a	7,650 a	8,470 b	5,580 b	5,710 b	3230 a	2690 a

* Means within the same vertical columns followed by the same letter are not different from each other ($p = 0.05$).

† Wheat was rotated after corn and only the half rate was applied before planting wheat.

‡ Soil test recommended rate. No nitrogen was applied to soybeans or wheat.

§ Full rate was 13 mt/ha in 1978, 1979, 1981, and 1982, and 11 mt/ha in 1980. Potassium at recommended rate applied to all sludge plots.

TABLE 2.20. LEAF AND GRAIN CONTENTS OF NUTRIENTS AND HEAVY METALS IN CORN GROWN WITH COLUMBUS SEWAGE SLUDGE AT FARM SCIENCE REVIEW

Treatment	Leaf								Grain							
	N	P	K	Cd	Cu	Ni	Pb	Zn	N	P	K	Cd	Cu	Ni	Pb	Zn
								µg/g								
							1978									
Control	28800b*	2580b	2250a	1.08b	8.5b	0.1b	1.4a	32.3c	11400a	2020a	2120b	0.14c	2.1a	0.8a	--	15.7c
Fertilizer	31900a	2790a	2340a	1.82ab	10.0a	0.2ab	1.6a	42.2b	12100a	2160a	2580a	0.16bc	2.1a	0.5a	--	16.8bc
Sludge-Half Rate†	29400b	2800a	2570a	1.93a	9.3ab	0.2a	1.6a	47.7ab	11900a	2150a	2300ab	0.19b	2.1a	0.4a	--	18.2ab
Sludge-Full Rate†	30400ab	2700a	2380a	2.08a	9.2b	0.2ab	1.5a	49.9a	12200a	2210a	2330ab	0.25a	3.0a	0.7a	--	19.7a
							1979									
Control	--	--	--	--	--	--	--	--	13000a	2680a	3310a	0.17a	1.8a	1.0a	--	18.4b
Fertilizer	--	--	--	--	--	--	--	--	14000a	2750a	3090ab	0.15a	2.1a	1.0a	--	19.5ab
Sludge-Half Rate	--	--	--	--	--	--	--	--	13900a	2390ab	3070b	0.16a	2.0a	0.9a	--	20.6ab
Sludge-Full Rate	--	--	--	--	--	--	--	--	13700a	2110b	2950b	0.17a	2.2a	1.0a	--	21.5a
							1980									
Control	--	3070a	23200a	0.16b	7.6b	0.1a	8.3a	31.2c	--	2850a	3430a	0.02a	0.9c	0.2a	0.1a	15.8a
Fertilizer	--	2990a	25800a	0.20ab	8.9a	0.1a	5.3b	38.8bc	--	2530b	3100b	0.04a	1.3a	<0.1a	0.1a	16.2a
Sludge-Half Rate	--	3110a	23400a	0.24ab	8.0b	<0.1a	4.9b	50.3ab	--	2720ab	3330ab	0.04a	1.1b	0.1a	0.1a	16.5a
Sludge-Full Rate	--	3090a	24900a	0.28a	8.1b	0.0a	5.5b	61.2a	--	2710ab	3210ab	0.03a	1.2ab	<0.1a	0.1a	17.5a
							1981									
Control	--	3090a	20500a	0.11ab	5.7b	0.8a	1.8a	28.7c	10300c	2410a	3160ab	0.05a	1.2b	0.2ab	--	15.3ab
Fertilizer	--	2820a	21800a	0.10b	7.4a	1.2a	1.7a	35.1bc	12700a	2300a	2950b	0.03a	1.3b	0.1b	--	11.8b
Sludge-Half Rate	--	2830a	21900a	0.11ab	6.3ab	0.4a	1.5a	41.0ab	11000bc	2590a	3040ab	0.02a	1.4ab	0.3a	--	16.7a
Sludge-Full Rate	--	2760a	20300a	0.14a	6.9ab	1.2a	1.3a	48.8a	11300b	2710a	3210a	0.04a	1.5a	0.4a	--	16.6a
							1982									
Control	--	1480b	21500a	0.30b	3.6bc	0.3a	--	28.8b	--	--	--	0.09a	0.6b	0.7a	0.8a	12.8a
Fertilizer	--	2270a	22100a	0.36a	5.1a	0.2a	--	38.3a	--	--	--	0.10a	0.9ab	0.6a	0.8a	13.7a
Sludge-Half Rate	--	1990a	21000a	0.31b	3.2c	0.2a	--	30.3b	--	--	--	0.11a	0.9a	0.7a	1.0a	14.3a
Sludge-Full Rate	--	2160a	21000a	0.35a	3.9b	0.3a	--	38.9a	--	--	--	0.10a	0.9a	0.7a	1.0a	15.0a

† Half Rate = 6.5 mt/ha (5.5 mt/ha in 1980); Full Rate = 13 mt/ha (11 mt/ha in 1980).
* Means within the vertical columns followed by the same letter are not different from each other ($p = 0.05$).

unexpected since the Bray P1 soil test levels were above the sufficiency level of 15-20 μg P/g on all plots (Table 2.22). There were also no differences in leaf K and no consistent differences in grain K. Potassium was applied uniformly to all plots and there were no differences in exchangeable K in any year (Table 2.22).

Of the metals, there were consistent increases in leaf Zn and Cd with sludge application compared to the control and fertilizer plots, with the greatest Cd effects in 1978 where the corn hybrid used had much higher Cd uptake than hybrids used in subsequent years. Hinsely et al. (1982) have shown large differences in Cd uptake among genetic corn lines. Grain Cd and Zn from sludge-treated plots were also higher in 1978 than grain from the control or fertilizer plots. There was a trend in all years for leaf Cu from the fertilizer plots to be higher than the sludge-treated or control plots. The only possible explanation for this is that Cu may have been added with fertilizer and increased leaf Cu while Cu added with sludge may have been less bioavailable because of its strong binding with sludge organic matter.

Levels of all metals in corn leaves or grain were low for all treatments compared to concentrations that are found for corn grown in highly metal-contaminated soils.

Leaf and grain N concentrations were low in wheat grown on sludge-treated plots compared to the fertilizer treatment, and were not significantly different from those from the control plots (Table 2.21). The half-rate sludge treatment provided 77 kg N/ha of available N, while the full-rate treatment only provided residual N from sludge supplied to the previous corn crop (Table 2.18). However, wheat yields were higher from the sludge plots than from the control or fertilizer plots (Table 2.19). As previously explained, wheat yields from the fertilizer plots would probably have been higher, but this treatment caused considerable lodging and seed head shattering because of earlier maturity. Therefore, it is possible that wheat on the sludge plots was N-limited.

There were no differences in wheat leaf or grain metal contents except grain Cu which was lower with the sludge treatments than the control or fertilizer treatments.

There were no consistent effects of sludge application on nutrient or metal concentrations of soybean leaves or grain (Table 2.21).

Soil Analysis--

Soil pH; exchangeable K; Bray P1 available P; total N, P and K; and total and DTPA-extractable metals in soil each year are given in Table 2.22 for the control, fertilizer and sludge treatments.

There was a trend for higher soil pH on the sludge-treated plots. This was due to the addition of lime to half of the sludge plots in 1978. Even though liming did not significantly increase pH, and these plots were treated the same statistically, they did raise the mean pH

TABLE 2.21. LEAF AND GRAIN CONTENTS OF NUTRIENTS AND HEAVY METALS IN WHEAT AND SOYBEANS GROWN WITH COLUMBUS SEWAGE SLUDGE AT FARM SCIENCE REVIEW

Treatment	Leaf								Grain							
	N	P	K	Cd	Cu	Ni	Pb	Zn	N	P	K	Cd	Cu	Ni	Pb	Zn
	μg/g															
	Wheat (1979)															
Control	24600b*	2470a	--	0.35a	32.2a	6.9a	5.7a	24.9a	18600b	2040a	4010a	0.17a	18.0a	3.0a	6.5a	43.1a
Fertilizer	32000a	2570a	--	0.31a	27.3a	6.0a	7.2a	26.2a	22600a	1510a	4000a	0.16a	15.8a	2.3a	6.7a	50.4a
Sludge-Half Rate†	24400b	2960a	--	0.37a	31.9a	3.3a	8.2a	27.2a	19300b	1860a	3760a	0.24a	11.0b	2.2a	5.6a	45.2a
Sludge-Full Rate†	27000ab	3020a	--	0.35a	22.6a	2.8a	6.3a	25.1a	19200b	1780a	3860a	0.25a	9.8b	2.5a	6.1a	44.7a
	Soybeans (1981)															
Control	--	3860a	28000a	0.59a	9.3a	3.7a	1.6ab	38.5a	59300a	4700b	19900a	0.13a	9.1a	3.2ab	0.0a	43.0ab
Fertilizer	--	4050a	27000a	0.21a	7.7b	5.0a	1.7a	35.2a	62700a	4680b	20400a	0.13a	5.7a	4.7a	0.0a	42.7ab
Sludge-Half Rate	--	4240a	27000a	0.85a	9.1ab	3.4a	1.4ab	38.4a	60300a	5320a	21000a	0.08a	8.6a	2.6b	0.0a	40.6b
Sludge-Full Rate	--	4210a	27000a	1.43a	8.1ab	3.5a	1.2b	45.4a	60300a	5220ab	21000a	0.15a	8.7a	3.2ab	0.2a	50.9a

* Means within the vertical columns followed by the same letter are not different from each other ($p = 0.05$).

† Half Rate = 6.5 mt/ha; Full Rate = 13 mt/ha.

TABLE 2.22. SOIL pH AND NUTRIENT AND METAL CONTENTS OF SOIL BY YEAR AFTER TREATMENT WITH COLUMBUS SEWAGE SLUDGE AT FARM SCIENCE REVIEW

Treatment	pH	Exchangeable		Total								DTPA Extractable				
		K	Bray P1	N	P	K	Cu	Cd	Pb	Ni	Zn	Cu	Cd	Pb	Ni	Zn
		μg/g														
							1978									
Control	5.7a*	--	33.8c	1708b	638a	5800a	17.5a	0.40b	32.2a	24.1a	82.2a	2.5b	0.16b	3.1b	1.3b	4.2c
Fertilizer	5.2b	--	39.0bc	2220a	786a	6377a	20.9a	0.56a	33.0a	22.3a	95.4a	3.6a	0.23a	3.6a	2.6a	5.3bc
Sludge-Half Rate†	5.5ab	--	50.6ab	1718b	665a	5481a	18.8a	0.44b	34.6a	22.6a	89.7a	2.7b	0.18ab	3.2b	1.3b	6.0ab
Sludge-Full Rate†	5.5ab	--	55.9a	1661b	705a	5293a	17.6a	0.44b	32.9a	20.8a	86.7a	2.7b	0.19ab	3.4ab	1.3b	7.4a
							1979									
Control	6.0a	155a	26.7b	1788a	632a	5712ab	20.5a	0.27a	16.1a	20.5a	91.0a	2.1a	0.16a	1.8a	2.2a	4.5ab
Fertilizer	5.9a	167a	28.4b	1978a	692a	6109a	20.4a	0.25a	16.4a	21.4a	86.0a	2.3a	0.16a	1.9a	2.3a	2.9b
Sludge-Half Rate	5.8a	153a	41.7ab	1703a	640a	5040b	20.6a	0.38a	17.3a	19.5a	92.8a	2.5a	0.21a	2.1a	1.9a	6.8a
Sludge-Full Rate	6.0a	152a	54.0a	1657a	660a	5057b	20.5a	0.41a	17.5a	18.8a	92.8a	2.4a	0.22a	2.0a	1.8a	8.1a
							1980									
Control	6.3ab	175a	26.2c	--	--	--	--	--	--	--	--	2.9a	0.21b	2.7b	1.9a	5.6c
Fertilizer	5.9b	187a	30.8c	--	--	--	--	--	--	--	--	3.3a	0.22b	3.0b	2.2a	6.1c
Sludge-Half Rate	6.5a	179a	56.9b	--	--	--	--	--	--	--	--	4.3a	0.33b	3.1ab	1.8a	10.4b
Sludge-Full Rate	6.5a	176a	79.1a	--	--	--	--	--	--	--	--	4.3a	0.43a	3.5a	2.3a	15.7a
							1981									
Control	6.2a	174a	27.9b	--	--	--	--	--	--	--	--	2.9b	0.25b	2.7b	1.7a	4.4b
Fertilizer	6.2a	168a	35.8b	--	--	--	--	--	--	--	--	3.5ab	0.26b	2.7b	1.7a	4.1b
Sludge-Half Rate	6.5a	173a	89.0a	--	--	--	--	--	--	--	--	4.5a	0.48a	3.1ab	1.6a	14.2a
Sludge-Full Rate	6.5a	171a	85.5a	--	--	--	--	--	--	--	--	4.6a	0.54a	3.3a	1.5a	13.1a
							1982									
Control	6.2a	260a	32.6c	--	--	--	15.1a	0.85bc	2.8a	16.2a	68.3b	2.6b	0.27b	2.8b	1.9a	5.8bc
Fertilizer	4.8b	240a	34.2c	--	--	--	15.2a	0.81c	2.8a	16.4a	63.8b	2.8b	0.24b	2.8b	2.3a	3.4c
Sludge-Half Rate	6.3a	225a	54.3b	--	--	--	16.7a	1.02ab	3.0a	16.0a	80.4a	3.2b	0.39b	3.0ab	1.6a	9.6b
Sludge-Full Rate	6.4a	237a	89.9a	--	--	--	17.2a	1.15a	3.3a	15.6a	86.8a	3.9a	0.55a	3.4a	1.8a	14.4a

* Means within the same vertical columns followed by the same letter are not different from each other ($p = 0.05$).

† Half Rate = 6.5 mt/ha (5.5 mt/ha in 1980); Full Rate = 13 mt/ha (11 mt/ha in 1980).

for the sludge plots compared to the control and fertilizer treatments. Also, digested sewage sludges have a tendency to raise pH's of acid soils.

There was a consistent significant increase in Bray P1 phosphorus with sludge and fertilizer treatments. By 1980, sludge additions had raised available P levels above that for the fertilizer treatment, and the full rate of sludge had higher levels than the half rate in 1980 and 1982.

Total nutrients (N, P, K) in soil (measured only in 1978 and 1979) were generally unaffected by fertilizer or sludge additions. This is not unexpected since this fertile till soil has high background levels of these three elements. Increases in total metals in soil with sludge treatment were not noted until 1982 and only with Cd and Zn. This points out the difficulty in using soil analysis to monitor metal accumulations when low rates of sludge-borne metals are applied. DTPA-extractable metals except Ni increased with the full rate of sludge application after 1980, although there was a consistent increase in DTPA-Zn from the first year of sludge application. DTPA did not appear to be sensitive to Ni additions.

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SECTION 3

SOCIOLOGICAL EFFECTS OF LAND APPLICATION OF SLUDGE

Robert E. Brown, M.S., P.E.
Ohio Farm Bureau Development Corporation
Columbus, Ohio 43216

Albert R. Pugh, M.S.
Cooperative Extension Service
The Ohio State University
Columbus, Ohio 43210

The sociological aspect of the sewage sludge project was one of the important keys to the success of the total project. Attitudes of Ohio residents regarding land disposal of municipal sewage sludge were suveyed by Musselman et al., 1980. They found that educational meetings were effective in changing residents attitudes and increasing acceptance of land application. However, general interest in the issue and attendance of public meetings on the issue is low unless a land application program is imminent. The issues of concern were potential odor problems, impacts on property values, and health and environmental risks.

The demonstration aspect of this project was to present the practicality of management systems which would overcome concerns of the rural community and still provide municipalities with reliable disposal sites for sludge. Sites were selected which would include the four major soil regions of Ohio and which would permit the involvement of both large cities and smaller water treatment systems. The demonstration areas included Franklin and Pickaway counties which received sludge from the city of Columbus; Clark county which received sludge from the city of Springfield; Defiance county which received sludge from the city of Defiance; and Medina county which received sludge from a county waste water treatment system. It was our desire to show that sludge management systems could address in an appropriate manner the concerns of rural residents under conditions which were typical of most of the state of Ohio.

The first step in initiation of the project was to present its scope to as many people in each community as possible. Detailed discussions were held between project staff and elected officials. Public meetings, radio, television, newspaper articles, field demonstrations and personal contacts were used to present the project to the general public. Almost every farmer in each community was contacted and invited to become involved in the project.

Organizations and officials which were involved included the Ohio Municiapal League, The Ohio County Commissioners Association, The Ohio Environmental Protection Agency, the Ohio Department of Health, County Boards of Health, Mayors, County Commissioners, City Managers, and County Farm Bureau Boards.

Legal Considerations Regarding Land Application of Sludge

A model contract to be used by farmers who receive sludge and the municipalities which generate sludge was drafted by the Ohio Farm Bureau Federation and the four project communities, (See Attachment). The contract is an attempt to carefully define the rights and responsibilities of the sludge generators and the farmers, and included the following priciples:

1. The Ohio State University Cooperative Extension Bulletin 598 is the authoritative document regarding appropriate management practices for land application of sludge.
2. Sludge is to be delivered and spread on farms by the communities generating the sludge without charge to the farmers.
3. The farmer and the community will mutually agree on the specific site where the sludge will be applied.
4. The farmer may terminate application activities if he believes that weather and field conditions are not suitable or that the application will result in damage to the field or crops.
5. The generating community will be responsible for monitoring the quality of the sludge and will supply the farmer with data on the composition of the sludge.
6. Application rates are to be mutually agreed upon by farmers and generators of the sludge.
7. The sludge must be well stabilized and free of offensive odors.

It was not possible to obtain a clause with our cooperating committees which would recognize the sludge generators as responsible for any liabilitites resulting from the application of sludge to the land. Rather it was agreed that this liability question would have to be decided on a case by case basis if and when problems arose.

Other clauses that may be included in a contract which are desirable for the protection of landowners include the following:

1. Indemnification. An agreement should provide that the city will indemnify a landowner for any expenses incurred by the landowner, such as attorney fees and damage awards, as a result of participation in a sewage sludge disposal project. The disposal of sewage sludge upon land may lead to litigation based upon such things as nuisance, water pollution and negligence. Unfortunately a landowner could be named a defendant in such litigation and held liable for damages. An indemnification clause will place the ultimate responsibility for payment of such damages upon the city.

2. Damage, Insurance and Performance Bonds. An agreement should provide that the city will compensate the landowner for any damages resulting from sludge disposal and it may provide that the city will keep insurance and a performance bond in force that covers liabilities arising under the agreement as well as other liabilitites associated with the disposal of sludge. The insurance and bond are a source of funds with which to pay the landowner and/or other persons damaged by sludge disposal activities. This is especially important when it appears that a city may not have the funds available to cover such damages.

3. Arbitration. An arbitration clause can help settle many disputes and avoid litigation and should be included in a sludge disposal agreement. Under arbitration, arbitrators chosen by the landowner and the city, would settle disputes arising under the agreement. This would frequently result in faster and cheaper resolutions of conflicts. In addition, it helps put the landowner in an equal bargaining position with the city because it avoids placing the landowner in a situation where he must hire an attorney to represent him.

Securing Cooperating Farmers

The recruitment of farmers for the project was initiated with letters from the County Extension Office inviting people out to a meeting regarding the project. Post cards were enclosed in the letters and were to be returned by the farmers to indicate if they planned to attend the meeting. A telephone campaign was then conducted to urge return of the post cards and attendence of the meeting. This generally resulted in an attendance of 50 to 100 people of which about one half were willing to become involved in the study. Recruitment of additional people varied with the community and included all modes of advertisement plus considerable one on one conversations between farmers and County Extension staff.

Those volunteering for the project were invited to a second meeting in the County Extension office for additional information. This provided all participants an opportunity to meet the researchers involved in the project and another time for questions and answers prior to the initiation of the project.

The educational phase of the project was conducted as objectively as possible. No attempt was made to " over sell" the concept of recycling of sewage sludge because this would only lead to mistrust of the participants. All of its facets were presented in an unbiased and professional manner.

Management of Land Application of Sludge

The demonstration method was used to present what leaders of the rural community would consider to be a well managed sludge disposal system. The objective was to minimize negative impacts on the rural community and still provide practical reliable conditions for municipalities involved in disposal of sewage sludges.

An odor problem was recognized as the most likely factor producing a negative impact. It was the position of the project directors that unstabilized, foul smelling sludge would not be applied to the land of cooperating farmers. If unstabilized sludges were to be applied to the land in an emergency, the sludge was to be injected into the soil.

A prime consideration was that no farm would be labeled as "the sludge disposal farm" with all the implied negative connotations. Instead as many farms as possible were to be involved in the project, each receiving a limited quantity of sludge on a small acreage. This practice would limit the sludge spreading activity in a given area to at most two weeks and no rural residents would be faced with the prospect of living next door to a sludge disposal site on a year round basis. This practice would also insure that any nuisances would be of short duration in a given neighborhood.

The project was concerned about environmental problems such as: runoff, water pollution, and metal accumulations. Control measures were carefully planned and implemented. Since sludge application was based upon the fertility needs of the crop, the amount of sludge applied was low. As a result the likelihood of sludge having an impact upon surface water or ground water was also reduced. The application rate varied from 4 to 10 dry metric tons per hectare. At these rates, sludge could be applied to a given field for twenty years or more without approaching the recommended limits for accumulation of metals.

Finding disposal sites during inclement weather was the most difficult management factor. During summer applications were made upon hay and pasture fields and wheat stubble. Fall, winter and spring applications were made upon corn and soybean fields. In all areas the relatively level topography permitted applications in the winter on frozen grounds. Applications were generally not possible when the ground thawed during the winter and during peroids of heavy rainfall in the spring. A capacity of up to two months storage of sludge at the sewage plant is essential in coping with periods of unfavorable field conditions.

Dairy farms were excluded from the project as recommended by the Ohio Department of Health. Applications were made on a sod farm in Franklin county.

Educational activities

The interest of farmer participants in the project was maintained by a number of methods. A newsletter was employed to inform participants of activities and accomplishments. Public meetings, field days, and tours were provided. The Cooperative Extension Service maintained close contacts with participants and also those sanitary officals involved in the spreading of the sludge.

Educational meetings were also sponsored for the benifit of sanitary engineers, municipal officials, and public health officals . Workshops were held annually since 1980 for the following people:

- County and municipal engineers and sewage treatment plant operators
- Consulting engineers
- Local government officials and health department personnel
- Agency personnel. Cooperative Extension Service, Ohio Environmental Protection Agency, USDA Soil Conservation Service, Ohio Department of Natural Resources Division of Soil and Water Conservation Districts, etc.

The workshop program was designed to cover equipment and system selection, soil properties and potential problems, health concerns, economic aspects, educational and public relation programs, and regulations. Other topics covered were contract hauling/spreading and composting of sewage sludge. Each workshop has included problem sets where the participants analyzed case situations and presented their conclusions before the workshop. Between 80 and 100 individuals have attended each year.

PROJECT DETAILS BY COUNTY

CLARK COUNTY

Clark County was the last county to be added to the research project. Clark County is located in West Central Ohio which has a population of 150,236 and the City of Springfield with 71,000 people. Springfield is a manufacturing city. Extensive efforts in pretreatment of waste by local industry has resulted in a suitable sludge. The city has been spreading sludge on farm land for several years and had good rapport with the area farmers.

John W. Kame, Clark County Extension Service office was selected to work with the project. He made the contacts with the farmers and worked at all the meetings and with the Springfield Waste Treatment Plant. Mr. Kame knew most of the farm families which aided in securing cooperators. The farms were in constant contact by phone or personal visit from Mr. Kame to keep them informed and to resolve any problems.

Farms in Clark County average 211 acres which is about average for the state. Clark County is No. 1 in beef cattle, but grain crops such as corn, soybeans, wheat, and oats are produced along with forage crops.

With the smaller farm near homes, special care was taken to keep the odor problem under control. During the whole project, there was not one complaint from a neighbor or citizen about the spreading of sludge.

The same educational program was conducted as outlined in the introduction. Additional meetings such as sludge farm tours each year and newspaper articles were used to keep the public informed.

Michael E. Haubner, County Extension agent, Agriculture and Community and Natural Resource Development, reported the following conclusions about the project in Clark County:

1. Everyone who participated in the project wants to continue or begin to receive sludge for their farms. All felt it was beneficial, saved them dollars and improved the tilth of their soil.

2. There were no complaints from anyone in Clark County about the spreading of sludge.

3. Everyone in the county was very positive about sludge being applied to farmland.

4. There were two main factors responsible for this positive, smooth running project.

   a. Mr. Jack Kame spent many hours working with cooperators and waste treatement plant officials to solve any immediate or foreseeable problems.

   b. The professional waste treatment plant officials ran an efficient, responsible operation.

5. Everything concerning this project was positive and land application of sewage sludge when carefully monitored can be very beneficial to local governments and farmers.

COLUMBUS PROJECT

The Columbus project was initiated as described above. Farms in the area are involved in the production of corn, soybeans, wheat and hay crops. A number of livestock farms are located in Pickaway County.

The Columbus project has been successful in Franklin County where Columbus is located. Sludge has been applied to Franklin County farms for over three years without any complaints from neighbors to the sludge receiving farms. It is important to note that strip housing was in the vicinity of the sludge spreading operations but not as close as in the Medina project.

The Pickaway County portion of the project was also operated successfully for over one and a half years. However,problems were encountered when a poorly stabilized sludge was applied to Pickaway County farms which were not involved in this project. As an emerengcy measure, Columbus obtained agreements with farmers who were not in the OFBDC project to receive a lime stabilized sludge cake. The sludge was placed in stocklpiles on fields in anticipation of being spread within two weeks. However, the weather changed making the spreading of sludge stockpiles impossible for a period of six weeks or longer. In some situations the odors became unbearable.

This incident resulted in the end of our Pickaway County project. Many non farm residents who live in strip housing developments in Pickaway County were suddenly stirred and opposed to further spreading of Columbus sludge within the county. Public meetings were conducted by the project staff to try to distinguish between this emergency of Columbus and the project sponsored by the OBFDC. It was found to be simply impossible to communicate with those opposed to sludge applications. Petitions were passed against the project and finally the local Board of Health placed an injunction against all spreading of sludge from Columbus within Pickaway County.

This situation reminded the residents of Pickaway County of the many conflicts of interest between the City of Columbus and the people in Pickaway County. Most of the farmers who had been involved in our project were willing to continue with the project. However, they were unable to prevail over the will of the non farm residents of the county.

It is important to note that no complaints were received from Franklin County residents even though the same lime treated sludge was applied in Franklin County.

It was the hope of the project staff that problems of this kind could be resolved through negotiation if they were encountered. In this particular incident the project was not responsible for the application of the odorous sludge, and we obtained assurances from the City of Columbus that the situation would not be repeated. These factors were presented to the local Board of Health and we received assurances that the situation could be resolved if we would work with them in reassuring the public of the credibility of the OFBDC project. As a result of their initial spirit of good will, we chose not to appeal the injunction in the courts. After a year of effort, the Board of Health did not lift the injunction. Our legal position was considerably weakened since the right to appeal was given up in initial talks. Further legal action was considered, but there was not sufficient time left in the project to make a court action meaningful. In the end the project in Pickaway County had to be abandoned because of the pressure of the local resident on the local Board of Health.

George Hamrick, County Extension Agent, Agriculture, Pickaway County, reported there were many farmers in the county who felt strongly about and would support the program, but the majority of the homeowners were against it. There appeared to be no one interested in taking the leadership locally for the continuation of the project.

Thomas J. McNutt, County Chairperson, reported that the sludge project tended to get general acceptance of the Franklin County people. Neighbor complaints were minimal and were handled by personal contact. Other problems encountered in Franklin County were minimal. Field storage accessibility due to weather and fall plowing seemed to be the biggest problem.

MEDINA COUNTY

Medina County located in the Northern Ohio suburban area south of Cleveland was the first county to participate in the project.

County-wide problems were not encountered due to an open and forthright relationship with the local news media.

The Extension agent reported that educational programs, good management, and the close working relationship with the Sanitary Engineers were major reasons for the success of the land application of sludge program. They laid the groundwork for a good land spreading program.

Those involved with the program felt good management went beyond delivering and applying sludge satisfactorily. Establishing a good rapport with each farmer, working with him, and earning his trust and respect, makes the difference between a mediocre or greatly successful program.

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CONTRACT

THIS CONTRACT, made this _____ day of _________, 19__, by and between __________________, hereinafter referred to as Owner, and ________________, hereinafter referred to as City, witnesseth that,

WHEREAS, Owner is the owner of a parcel of agricultural real property located in ________________, ________________, ____________________, Ohio,

(Parcel No.) (Township) (County)

which can be reached as follows: ____

____

____

____,

and

WHEREAS, City operates a waste treatment disposal plant which after processing produces a product known as sewage sludge, and

WHEREAS, Activities contemplated under this Contract are to be undertaken as a part of a project coordinated by the Ohio Farm Bureau Development Corporation under a grant entitled "Demonstration Program to show Ohio Landowners and Municipalities acceptable systems for applying Sludge on Land" from the United States Environmental Protection Agency,

WHEREAS, Owner will allow sewage sludge from City to be placed on the above mentioned real property only on the terms set out below,

NOW THEREFORE, Owner and City mutually agree as follows:

1. The "Ohio Guide for Land Application of Sewage Sludge", Bulletin 598 of the Cooperative Extension Service of the Ohio State University, as revised in May, 1976, shall be used as a guideline for responsible management practices. Hereinafter Bulletin 598 will be referred to as "The 1976 Guide."

2. The City will deliver sewage sludge to the above mentioned property of Owner and will properly spread or otherwise deposit said sewage sludge on said property without charge to the Owner. City shall be responsible for equipment used to deliver and spread such sewage sludge.

3. The Owner and the City will mutually agree on the specific portion of said property which is to receive sludge. In the absence of unusual factors, they will abide by the site selection criteria of The 1976 Guide.

4. The Owner or his representative may decline to receive sludge on said property when, in Owner's or his representative's judgement, the sludge application equipment would damage the soil structure because of excessive soil moisture at the disposal site. When possible, the Owner will give the City notice of poor field conditions 24 hours prior to the appointed application time. However, the City does realize that this is not always possible and that there will be some days when untimely excessive rainfall will require termination of spreading activities at a moment's notice on a given field.

5. The Owner will notify City in writing of the dates between which City may deliver and spread sewage sludge. The City may deliver said sewage sludge only during the period thus described. The Owner will make himself or his representative available to City or its employees during such period to ensure said sewage sludge is deposited on the proper location on said property.

6. Owner shall specify the access to be used by the City when sewage sludge is applied to a specific portion of said property. The Owner shall provide and maintain an access for use by the City without charge to the City, and the City shall not be liable for any damages thereto, except damage caused by City's negligence.

7. The Owner and the City will mutually agree on the rate or in what amounts per acre said sewage sludge is to be applied in a given year. They will also use the criteria of The 1976 Guide to determine the maximum total amount of sewage sludge per acre that can be applied to a given field.

8. The City shall properly analyze its sewage sludge on a monthly basis for the total nitrogen, ammonia and nitrate nitrogen, phosphate, potassium, lead, zinc, nickel, copper, and cadmium content. The results of such analysis will be provided to the Owner or his representative upon request without charge before sludge is applied to said property.

9. City shall keep and maintain records of the following items, and shall make such records available to Owner or his representative upon request:

(a) All analyses of the composition of sewage sludge produced by the City.
(b) All reports concerning the operation or production of sewage sludge by the City.

(c) All applications to agricultural land of sewage sludge produced by City including dates of application, amounts applied, specified rates of application, specific parcels of land upon which sewage sludge has been applied.

(d) All required governmental permits or approvals for the application of sewage sludge on agricultural land.

10. City will deliver and apply sludge which is well stabilized and which does not present a severe odor nuisance to Owner or other rural residents who live in the vicinity of the sludge disposal site. The Owner may refuse to accept any sludge which is exceptionally odorous.

11. This Contract shall continue in effect for a period of one year following the date first above written. The Parties hereto may renew this written notice to the other party of the intention to do so. Cancellation will be effective five days after receipt of such notice. Such notices shall be delivered personally or by certified mail to the address(es) listed at the end of this Contract.

OWNER:	CITY:
______________________	______________________
______________________	By. ______________________
Address: ______________________	Title ______________________
______________________	By ______________________
	Title ______________________
	Address: ______________________
	______________________

SECTION 4

SOIL COMPACTION WITH SLUDGE APPLICATION

Richard K. White
The Ohio State University
Columbus, Ohio 43210

This phase of the study of the effects of land application of sludge was undertaken because of concerns expressed by some individuals that heavy loads of sludge carried by large, heavy spreading equipment would cause serious soil compaction problems that would exist for long periods of time. Soil scientists have shown that in seriously compacted soils the movement of water and dissolved nutrients is decreased; the soil porosity (air space) is decreased, restricting oxygen availability to roots and hindering plant growth and development; and the increased soil bulk density increases draft and energy requirements for tillage operations. While numerous samples (over 1,300) from five farms were analyzed for bulk density and moisture, and three times that number were measured for penetration resistance, most of the detailed study was performed using soil samples from the three farms with compaction-related factors described in Table 4.1 (See Table 4.1).

Ideally, for a detailed research study the various types of application equipment would have been compared using compaction data taken from common fields (soil types) where they all operated at the same time. As this was not feasible in this project only compaction effects of a particular sludge spreading machine on a particular soil type could be examined. Sludge application rates (tons/acre) in any given field were held to fairly low levels also since the overall project was designed to test the feasibility of sludge application on normally productive farm lands.

TABLE 4.1 FACTORS RELATED TO SOIL COMPACTION ON THREE FARM STUDIED

	Knoch Farm Medina	Hoshock Farm Defiance	Hastings Farm Pickaway County Columbus
Sludge Application Equipment	Bovie	1HC 1056 Tractor Badger Tank Wagon	Terragator Model 2505
Approx. Gross Wt. Lbs.	20,500	42,000 (both)	24,000
Soil Type	Wadsworth Silt Loam	Hoytville Silty Clay	Miami Silty Clay Loam
Sand %	21.4%	16.5%	7.3%
Silt %	63.2%	43.6%	59.2%
Clay %	15.4%	39.9%	33.5%
Average Moisture % Retention at 15 atm (0-12 in.)	7.7%	17.6%	14.3%
Plastic Limit, Avg. % (0-12 in.)	18.8	23.2	22.6
Liquid Limit, Avg. %	29.1	51.8	45.2
Shrinkage Limit, % (0-12 in.)	19.4	15.4	17.8
Volumetric Shrinkage %	28.3	81.3	56.4
Crops Grown	Corn	Hay, Corn	Pasture

Objectives of the Compaction Study:

The soil compaction phase of this study had as its objectives:

1. To determine if sludge spreading equipment caused any significant soil compaction effects when used as it was in this study.
2. To determine the effects of subsequent plant growth, natural settlement, and/or winer freezing and thawing in ameliorating any machinery compaction actions experienced.
3. To compare compaction effects at different depths below the soil surface to determine the region where most damage can occur.

4. To relate soil bulk density to soil moisture content so as to identify moisture zones wherein maximum soil compaction is likely to occur.

5. Penetrometer readings and/or soil moisture values to predict soil bulk density.

Procedure:

For the areas being tested there were soil types varying from clay to silt loams and an assortment of application equipment ranging from a tractor pulling a tank wagon at Defiance to heavy equipment with large flotation-type tires near Columbus. The sludge applied ranged from liquid to semi-solid in nature, and application to fields occurred at any time during the year. It is well known that compaction effects are minimal when soil is frozen or when it is dry, so the critical application times were during the spring, summer, or fall when the soil was at a critical moisture level and in a plastic state.

At each farm area, maps were sketched showing the exact locations of control areas (no sludge applied) and of "transects" across the vehicle tracks, where readings and samples were taken. A transect required that soil core samples be taken as follows: one sample was taken outside each of the two tire tracks; two samples were taken between the two tire tracks; and three samples were taken in each of the two tire tracks. Thus, ten core samples were taken for each transect. Penetrometer readings were taken so that three penetrations were made near every core sample location. For each sludge application, four separate transects were taken for statistical replication. Therefore, a total of 40 core samples and 120 penetrometer readings were taken each time at each site. In addition, 10 sets of readings were taken from the control area during the same test period. In the laboratory the 24-inch soil cores were analyzed to determine moisture content and bulk density at 3, 9, 15, and 21-inch depths.

In the analysis of data, contour plots of soil profiles under the transects were made to examine soil moisture and density distributions to determine any compaction effects at various depths. Statistical comparisons of readings from transects and those from the control areas were made to see if significant differences were found between treated and untreated areas. Graphs of bulk density vs. soil moisture were plotted to determine the critical moisture levels in the soil when operation of heavy sludge spreading equipment would be most detrimental. This is known as the Proctor density test in the laboratory as used by highway and construction engineers.

Conclusions:

1. At the Knoch farm the differences in bulk density between readings taken under wheel tracks and those taken in undisturbed soil were significant at the 12-18 inch level after the second and third applications of sludge. No significant differences were noted in the 0-6 and 6-12 inch layers. This could indicate that compaction effects of heavy equipment in the upper 10-12 inches of soil can be obliterated by subsequent

tillage operations, but soil at lower depths retains the compaction effects. Winter freezing and thawing effects were negligible in soil under wheel tracks as in soil out of wheel tracks.

2. At the Hoshock farm, soil compaction under wheel tracks was significantly greater than on soil out of wheel tracks in the 6-12 inch soil layer. Average dry bulk densities were 1.78 gm/cc. in-track versus 1.72 gm/cc. out-of-track. It is not known whether this amount of bulk density increase would significantly affect crop growth. Penetrometer readings (cone indices) verified that penetration resistance under tracks was significantly greater than readings taken out-of-tracks.

3. At the Hastings farm the overwintering had a negative effect in some soil layers. Bulk density was significantly greater about 11 months after sludge application in the 12-18 inch layer under the tracks and in the 6-12 inch layer out-of-tracks. The explanation appears to be that cows were pastured in the area and their hooves created zones of compacted soil below the surface.

4. Laboratory tests of soils from the three farms indicated the critical moisture contents (percent dry weight basis) wherein maximum soil compaction occurred were as follows:

Layer (inches)	Knoch	Hoshock	Hastings
0-6 22% db	29% db	29% db	29% db
6-12	19% db	27% db	27% db
12-18	18% db	24% db	24% db
18-24	17% db	23% db	23% db

5. Pentrometer readings generally did not correlate well with dry bulk density values of samples taken from the various soil layers. However, when penetrometer readings and moisture contents were used jointly as variables to predict bulk density the highest correlation values were obtained. An equation of the form below resulted:

$BD + A + BM + CP + MDP$ where:

BD = Bulk Density, M = Moisture Content (db),

P = Penetrometer readings (cone index), and

A, B, C, D are constants

6. As an overall conclusion it appeared that soil compaction due to the sludge application equipment was not of great concern when applied in the quantities and frequencies of this study. If serious compaction ever does result, it will probably occur in the 12-18 inch layers.

SECTION 5

NITROGEN MINERALIZATION FROM SOILS AMENDED WITH SEWAGE SLUDGES

Terry J. Logan, B.S., M.S., Ph.D.
Robert H. Miller, B.S., M.S., Ph.D.
Patricia Lentz, B.S.
The Ohio State University
Columbus, Ohio 43210

INTRODUCTION

Objectives

Sludge application to land must be regulated to avoid harmful environmental effects, such as an accumulation of heavy metals and the leaching of excess nitrate into ground and surface waters. The nitrogen content of sludge is extremely variable and information on the broad range of variables is lacking. In order to avoid adverse effects on the surrounding environment as well as to determine reasonable supplemental nitrogen fertilization requirements for sludge-applied farmland, it is critical to know rates of N mineralization, percent sludge organic N mineralized, and how environmental, soil and sludge factors affect mineralization and nitrification.

Materials and Methods

The surface samples of five soils listed in Table 5.1 were mixed with acid-washed quartz sand (1:1 ratio dry weight basis). Liquid sludges from five treatment plants (Tables 5.2 and 5.3) were applied to each soil at a rate equivalent to 22.4 dry metric tons/ha. Each sludge (0.15 g dry weight) was thoroughly mixed with each soil-sand sample (30 g dry weight) to create all possible combinations of treatments, then packed into leaching tubes and wetted to field capacity. Glass wool was used beneath and above soil column to retain it and avoid disperson. Two replicates of each treatment plus controls were incubated at 25 C. Each tube was leached with a 100 ml aliquot of 0.01 M $CaCl_2$ at day 0, then weekly for an eight week period. Steam distillation (standard micro Kjeldahl) (Bremner, 1965) was used to determine extracted total inorganic N and NH_4-N in the leachate. Tubes were sealed with Parafilm between leachings.

TABLE 5.1. PROPERTIES OF THE SOILS USED IN THE MINERALIZATION STUDY

Property	Soil				
	Brookston	Crosby	Hoytville	Mahoning	Muskingum
Texture	Silt Loam	Silt Loam	Silty Clay Loam	Loam	Silt Loam
pH	4.8	4.7*	7.2	7.1	5.8
CEC (meq/100 g)	21	15	29	10	12
Base Saturation (%)	54.2	37.7	99.7	99.7	69.0
Bray P1 (µg/g)	30	28	23	20	12
Exch K (µg/g)	177	159	191	66	277
Exch Ca (µg/g)	1780	820	4815	1405	1285
Exch Mg (µg/g)	251	145	479	341	129
H_2O After Sludge Applied (%)	17.0	15.1†	11.6	18.2	21.1
H_2O at 1/3 atm. (%)	29.5	28.6	38.2	23.0	32.8
Total C (%)	2.25	1.50	2.37	1.20	2.56
Carbonate C (%)	0.00	0.00	0.28	0.24	0.00
TKN (µg/g)	2187	1545	2506	1357	2684
Organic N (µg/g)	2123	1485	2484	1334	2612
C:N Ratio	10.6	10.1	9.5	9.0	9.8

* The limed soil had a pH of 6.6.

† The limed soil had 21.0% H_2O after sludge addition.

TABLE 5.2. PROPERTIES OF SLUDGES USED IN THE 25 C INCUBATION STUDY

	Zanesville	Jackson Pike	Medina 100	Medina 300	Defiance
Digestion	anaerobic	anaerobic	aerobic	aerobic	anaerobic
Percent solids as applied to soil	5.8	5.3	6.5	7.6	9.7
pH	7.1	7.3	6.7	7.0	7.6
Total carbon (%)	17.9	30.2	28.8	23.4	24.7
Carbonate-C (%)					
Organic-C (%)					
C:N ratio	17:1	11:1	8:1	8:1	16:1
TKN (μg/g)	14,284	49,721	38,310	31,590	24,952
NH_4-N (μg/g)	3,738	21,645	3,675	2,531	9,649
Organic-N (μg/g)	10,546	28,076	34,635	29,059	15,303
Total P (μg/g)	10,223	21,973	19,975	27,025	33,253
K (μg/g)	6,497	4,142	8,337	8,688	6,407
Cd (μg/g)	225	56	8.8	8.3	28.9
Cu (μg/g)	552	677	615	738	387
Pb (μg/g)	3,132	403	432	389	601
Ni (μg/g)	54	291	42	45	1,212
Zn (μg/g)	2,622	4,153	903	1,340	2,047

TABLE 5.3. PROPERTIES OF SLUDGES USED IN THE 15 C INCUBATION, PH AND CD STUDIES

	Zanesville (August)	Jackson Pike	Medina 100	Medina 300	Defiance	Zanesville (February)
Digestion	anaerobic	anaerobic	aerobic	aerobic	anaerobic	anaerobic
Percent solids as applied to soil	11.1	5.0	5.3	5.6	6.5	---
pH	7.4	7.0	7.1	7.1	7.1	7.3
Total carbon (%)	32.2	31.3	28.9	17.7	28.8	20.3
Carbonate-C (%)						
Organic-C (%)						
C:N ratio	21:1	12:1	11:1	8:1	9:1	17:1
TKN (µg/g)	19,412	44,505	36,525	23,713	44,969	20,873
NH_4-N (µg/g)	3,977	17,324	9,202	484	14,263	8,677
Organic N (µg/g)	15,435	27,181	27,323	23,229	30,706	12,196
Total P (µg/g)	10,152	23,876	17,108	32,712	24,252	13,625
K (µg/g)	4,464	4,338	7,565	8,430	4,777	1,691
Cd (µg/g)	188	83.7	9.1	6.0	9.5	544
Cu (µg/g)	638	753	715	635	359	774
Pb (µg/g)	3,361	558	243	146	415	4,506
Ni (µg/g)	54	298	40	40	548	53
Zn (µg/g)	2,514	4,976	1,051	1,129	1,595	4,034

The preceding procedure was repeated at a 15 C incubation temperature, and basically for two other experiments with the following exceptions:

The Crosby soil was limed with $CaCO_3$ and freeze-dried Jackson Pike sludge was applied to both limed and unlimed soil samples. Freeze dried samples of high and low cadmium Zanesville sludges were added to the Muskingum soil. Three replicates of these additional treatments plus controls were incubated at 25 C.

RESULTS AND DISCUSSION

Mineralization

Cumulative net N mineralization, when plotted against the square root of time, in most cases yields a straight line (Stanford and Smith, 1972).

Data were plotted, a best fit curve drawn and the slope or mineralization rate of each treatment was estimated manually.

The data from replicates were averaged in most cases. However, in 14 of the 67 different treatments, only one replicate was used in calculating the data. The other replicate proved to be unrepresentative due to very low levels of net N mineralized. This can be attributed to imperfect packing and/or settling of the soil in the tubes so that one of two situations could occur. Either the leachate channeled through the tube too rapidly causing incomplete contact of $CaCl_2$ with the soil, leaving much of the NH_4^+ and NO_3^-; or anaerobic conditions prevailed in the tube bringing about substantial denitrification of mineralized and nitrified N. The fact that the sludge was added and mixed with the soil on an individual treatment basis also accounts for irregularities between replicates. Although the sludge was quantitatively added, the consistency of each sludge varied greatly. A uniform solids content of the liquid sludges could not be guaranteed for such minute amounts. The sludges which were least homogeneous were the Medina sludges. These situations could have occurred to a lesser extent in other tubes, although the similarity between replicates is reason to doubt their significance.

The data is given in Tables 5.4 and 5.5 and examples of N mineralization versus t 1/2 plots are given in Figures 5.1-5.3.

Sludge Effects

Few generalizations can be made concerning the relative mineralization rates of the soil-sludge treatments. In nearly all cases the rates were higher than the corresponding control. This is to be expected with the addition of an organic N source having a low C:N ratio. The treatments in which the mineralization rate was below

TABLE 5.4. NITROGEN MINERALIZATION RATE AND CUMULATIVE (8 WEEK) N MINERALIZATION INCUBATED AT 25 C (MEANS OF DUPLICATE TREATMENTS)

Treatment	N Mineralization Rate	Cumulative N Mineralized	Percent of Sludge Organic N Mineralized
	(µg N/g soil/wk)	(µg N/g soil/8 wks)	
Brookston control	26.2	52.7	—
Brookston + Zanesville*	47.8	103.1	46.0
Brookston + Jackson Pike*	60.1	132.9	28.2
Brookston + Medina 100	56.3	125.0	20.4
Brookston + Medina 300*	65.8	149.3	32.8
Brookston + Defiance*	31.8	73.7	13.5
Crosby control	16.6	32.1	—
Crosby + Zanesville	27.1	52.2	18.7
Crosby + Jackson Pike	38.9	90.3	20.6
Crosby + Medina 100*	49.2	120.6	24.7
Crosby + Medina 300*	44.4	107.4	25.6
Crosby + Defiance	30.2	79.8	31.1
Hoytville control	8.0	22.1	—
Hoytville + Zanesville	18.7	53.3	29.4
Hoytville + Jackson Pike	46.3	144.1	42.8
Hoytville + Medina 100	64.0	126.6	29.1
Hoytville + Medina 300	89.6	102.5	27.1
Hoytville + Defiance	8.2	24.0	6.2
Mahoning control	23.5	43.0	—
Mahoning + Zanesville	50.5	94.0	47.1
Mahoning + Jackson Pike	74.6	182.0	48.5
Mahoning + Medina 100	93.6	168.3	35.6
Mahoning + Medina 300	79.9	157.4	38.5
Mahoning + Defiance	15.0	35.8	—
Muskingum control	28.9	59.2	—
Muskingum + Zanesville	4.4	17.1	—
Muskingum + Jackson Pike	65.2	149.7	32.1
Muskingum + Medina 100*	39.3	52.7	—
Muskingum + Medina 300*	77.2	130.1	23.3
Muskingum + Defiance*	52.0	94.0	22.0

* Calculation based on one replicate.

TABLE 5.5. NITROGEN MINERALIZATION AVERAGES OF DUPLICATE TREATMENTS INCUBATED EIGHT WEEKS AT 15 C

Treatment	N Mineralization Rate	Cumulative N Mineralized	Percent of Sludge Organic N Mineralized
	(μg N/g soil/wk)	(μg N/g soil/8 wks)	
Brookston control	22.2	29.2	---
Brookston + Zanesville*	19.9	16.3	---
Brookston + Jackson Pike*	41.8	95.9	24.5
Brookston + Medina 100	64.3	113.6	30.6
Brookston + Medina 300*	59.9	66.4	16.0
Brookston + Defiance*	43.0	77.1	15.4
Crosby control	21.5	22.8	---
Crosby + Zanesville	21.4	23.1	---
Crosby + Jackson Pike	39.8	72.3	18.2
Crosby + Medina 100*	64.8	92.7	25.3
Crosby + Medina 300*	34.8	46.6	10.2
Crosby + Defiance	41.5	78.1	17.5
Hoytville control	15.0	19.7	---
Hoytville + Zanesville	6.5	13.7	---
Hoytville + Jackson Pike	57.9	111.1	33.2
Hoytville + Medina 100	62.6	108.3	32.4
Hoytville + Medina 300	31.4	49.3	12.6
Hoytville + Defiance	50.7	105.9	27.8
Mahoning control	27.1	27.4	---
Mahoning + Zanesville	9.9	17.9	---
Mahoning + Jackson Pike	73.6	127.7	36.6
Mahoning + Medina 100	79.8	135.1	39.1
Mahoning + Medina 300	38.6	46.5	8.2
Mahoning + Defiance	60.6	92.4	21.0
Muskingum control	23.8	29.4	---
Muskingum + Zanesville	11.2	14.4	---
Muskingum + Jackson Pike	53.8	88.7	21.8
Muskingum + Medina 100*	58.7	104.4	27.6
Muskingum + Medina 300*	59.1	56.8	11.5
Muskingum + Defiance*	57.8	84.0	17.3

* Calculation based on one replicate.

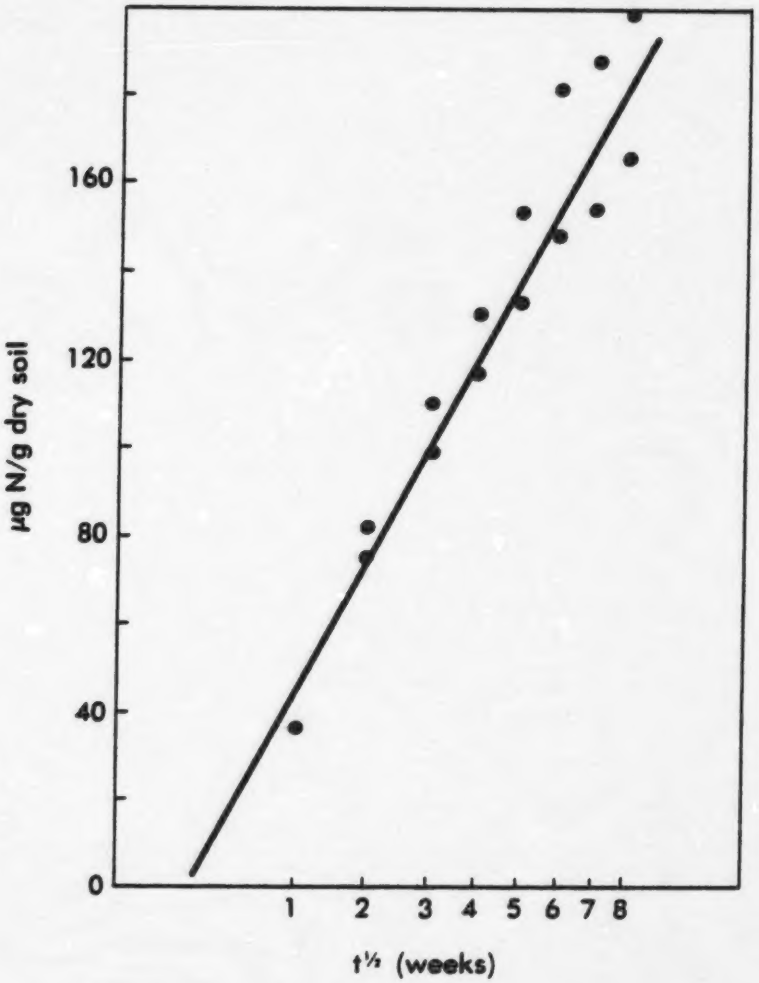

Figure 5.1. Cumulative inorganic N mineralized at 25C. Mahoning soil and Columbus sludge.

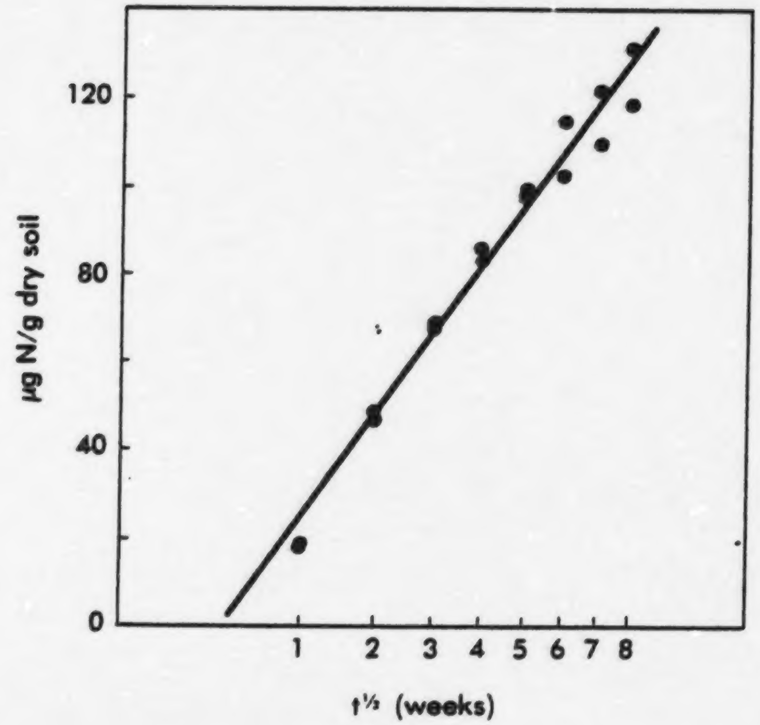

Figure 5.2. Cumulative inorganic N mineralized at 25C. Brookston soil and Medina 100 sludge.

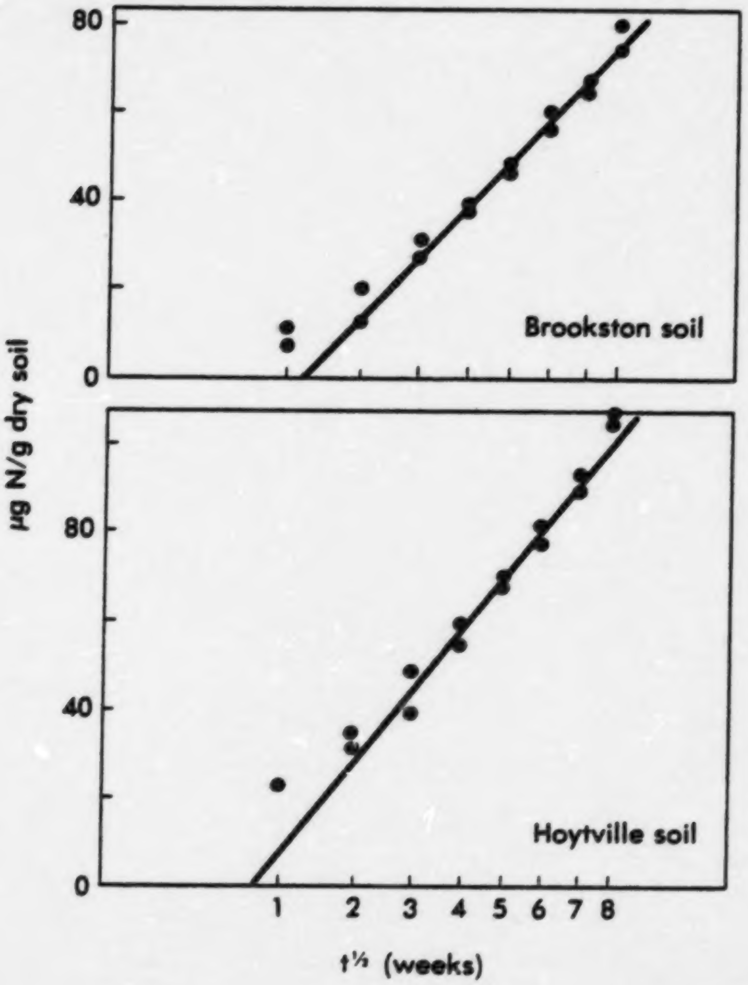

Figure 5.3. Cumulative inorganic N mineralized at 15C. Brookston soil and Defiance sludge (above); Hoytville soil and Defiance sludge (below).

that of the control are: 1) Zanesville for all soils incubated at 15 C; 2) Defiance sludge and Mahoning soil at 25 C; and 3) June, February and August Zanesville sludges and Muskingum soil at 25 C.

The generally lower rates for the Zanesville sludge treatments at 15 C could reflect a variety of circumstances. Relatively large quantities of cadmium and lead compared to the other sludges, the added stress of the lower temperature and a higher C:N ratio (21:1) of any of the sludges used, could have created a synergistic effect on inhibiting the ammonifying microorganisms. By far the most important factor in reducing net mineralization is the C:N ratio, which is sufficiently high to produce greater immobilization.

Low mineralization rates for Defiance sludge and Mahoning soil at 25 C reflect the relatively high C:N ratio of the sludge (16:1) coupled with a relatively low level of natural soil organic matter and organic nitrogen.

Mineralization rates for the high and low Cd Zanesville sludges and Muskingum soil at 25 C are quite interesting. As can be seen in Figure 5.4 in both cases, two of the replicates had low mineralization while the third was much higher. Lack of homogeniety of the mixed samples appears to be the cause of this inconsistency.

Figures 5.5 and 5.6 are examples of non-linear character. There are two possible explanations for this 1) The point where the rate drops off signifies that the readily mineralizable sources of N in the sludge were rapidly degraded by the microbial population. A peak was reached before the end of the incubation period; 2) at the lower temperature mineralized N differed very little in the first two to three weeks. Since this pattern was recurring the lower temperature seemed to induce a "lag" period before N mineralization progressed. These horizontal points were not used in determination of the slope of the lines because they were considered unrepresentative of the actual rate.

The data in Tables 5.6 and 5.7 reveal an inverse relationship between mineralization rates at 25 C and the C:N ratio of each sludge; i.e. the higher the mineralization rate, the lower the C:N ratio. At 15 C the same is true for all but Medina 300 which had the lowest C:N ratio (8:1), but also a rather low mineralization rate. From Tables 5.6 and 5.7, it is clear that the three sludges with the lowest C:N ratios mineralized the greatest amount of N with the exception of the Medina 300 (15 C). Less N was mineralized from Zanesville (C:N 21:1) than the control.

It can be seen from Table 5.8 that, for those treatments in which mineralization occurred, the average percent N mineralized varied between sludges at 25 C. Lower figures for the Medina sludges in comparison to Zanesville and Jackson Pike are accounted for by two

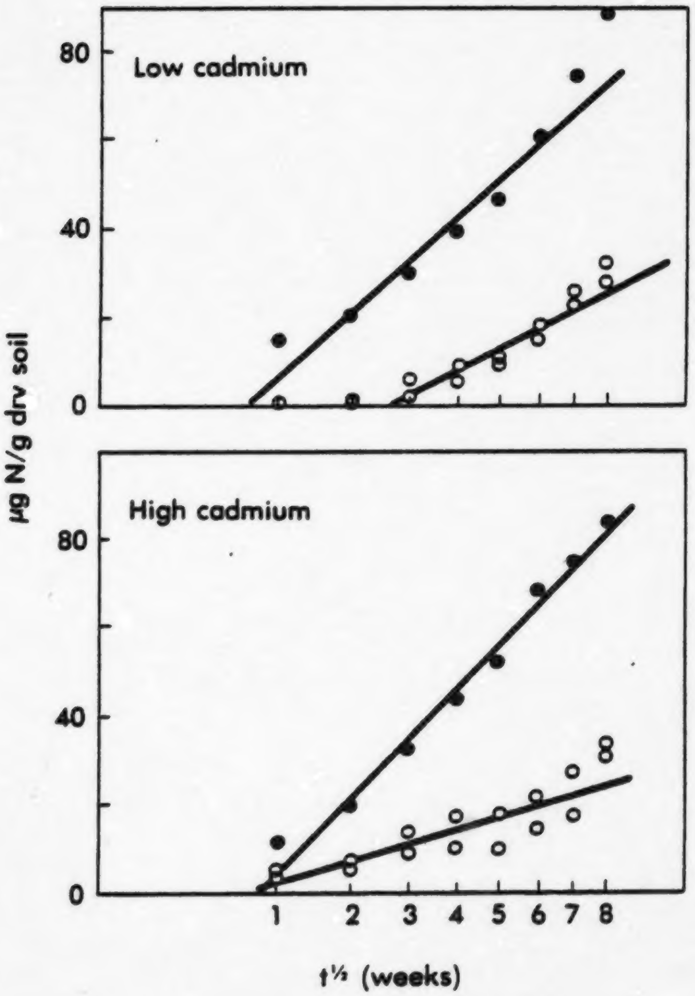

Figure 5.4. Cumulative inorganic N mineralized at 25C. Muskingum soil and low Cd Zanesville sludge (above); Muskingum soil and high Cd Zanesville sludge (below). Open circles represent one replicate, and open triangles are the other two replicates.

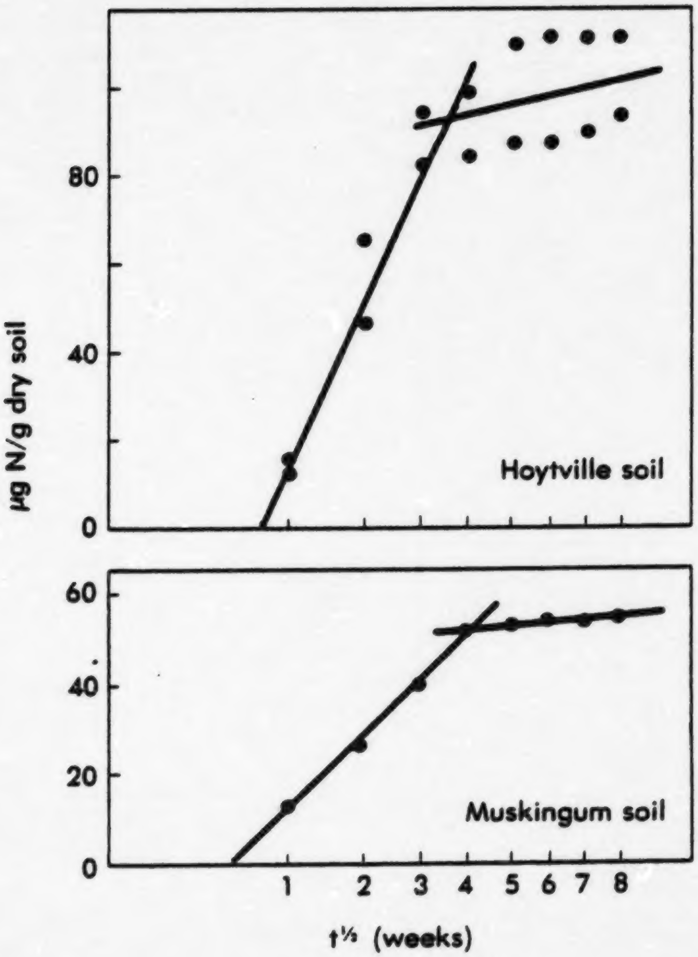

Figure 5.5. Cumulative inorganic N mineralized at 25C. Hoytville soil and Medina 300 sludge (above); Muskingum soil and Medina 100 sludge (below).

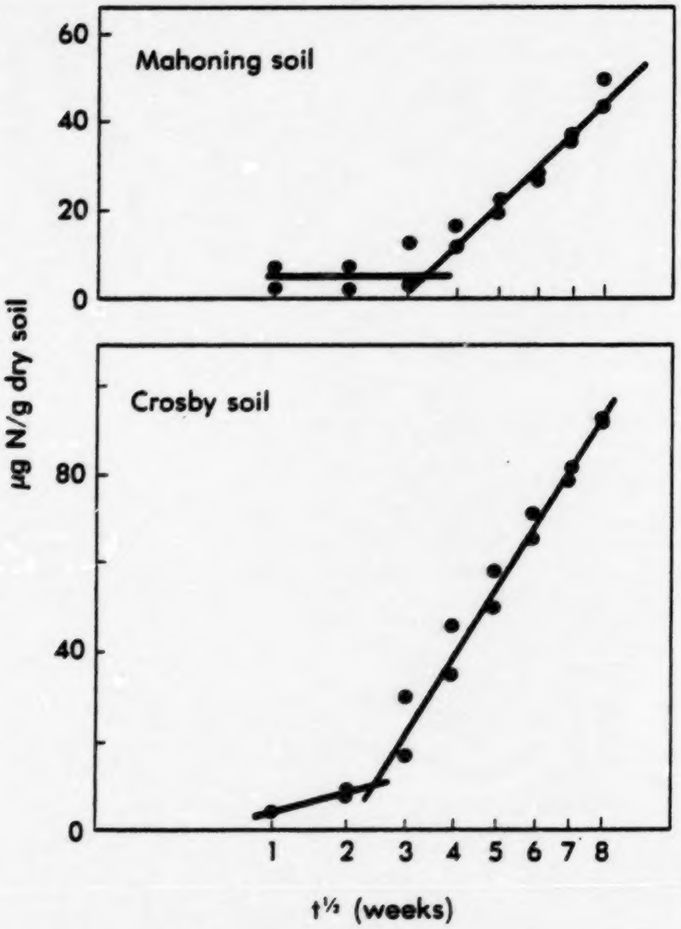

Figure 5.6. Cumulative inorganic N mineralized at 15C. Mahoning soil and Medina 300 sludge (above); Crosby soil and Medina 100 sludge (below).

TABLE 5.6. AVERAGE N MINERALIZATION RATES (μg N/g SOIL/WK$^{1/2}$) FOR SLUDGES INCUBATED AT 25 AND 15 C

Sludge	25 C	C:N	15 C	C:N
Zanesville	29.7	17:1	13.8	21:1
Jackson Pike	57.0	11:1	53.4	12:1
Medina 100	60.5	8:1	66.0	11:1
Medina 300	71.4	8:1	44.8	8:1
Defiance	27.4	16:1	50.7	9:1
No sludge	20.6		21.9	

TABLE 5.7. AVERAGE CUMULATIVE N MINERALIZATION (μg N/g SOIL) FOR SLUDGES INCUBATED AT 25 AND 15 C

Sludge	25 C	C:N	15 C	C:N
Zanesville	63.9	17:1	17.1	21:1
Jackson Pike	139.8	11:1	103.1	12:1
Medina 100	118.6	8:1	110.8	11:1
Medina 300	129.3	8:1	53.1	8:1
Defiance	61.5	16:1	87.5	9:1
No sludge	41.8		25.7	

TABLE 5.8. PERCENT SLUDGE ORGANIC N MINERALIZED IN 8 WEEKS

Soils	Zanesville	Jackson Pike	Medina 100	Medina 300	Defiance	Mean
25 C						
Brookston	46.0*	28.2*	20.4	32.8*	13.5*	28.2
Crosby	18.7	20.6	24.7*	25.6*	31.1	24.1
Hoytville	29.4	42.8	29.1	27.1	---	32.1
Mahoning	47.1	48.5	35.6	38.5	---	42.4
Muskingum	---	32.1	---	23.3*	22.0*	25.8
Mean	35.3	34.4	27.5	29.5	22.2	30.2
15 C						
Brookston	---	24.5	30.6	16.0*	15.4	21.6
Crosby	---	18.2	25.3	10.2*	17.5*	17.8
Hoytville	---	33.2	32.4	12.6	27.8	26.5
Mahoning	---	36.6	39.1	8.2	21.0	26.2
Muskingum	---	21.8	27.6	11.5*	17.3*	19.6
Mean	---	26.9	31.0	11.7	19.8	22.4

* Calculations based on one replicate.

facts: 1) Averaging did not include soils where net mineralization was below the control. 2) Although Medina sludges had a greater mineralization rate, an early peak was reached and points thereafter were not considered in the rate calculation. Lowest average percent N mineralized was for Defiance, again indicative of its high C:N ratio.

At 15 C with August sludges identical trends can be seen. Where C:N ratios are high (Zanesville), there was no percent N mineralized above the control.

Plots of Medina 300 show the longest, most pronounced lag period, hence the lowest percent organic N mineralized. The percent N mineralized in the remaining sludges corresponds to C:N ratios, mineralization rates and amount of N mineralized.

Soil Effect

Tables 5.9 and 5.10 summarize the general effects of surface soil properties on N mineralization. Mahoning loam gave the highest rates. Major contributing factors are an optimum pH (7.1), better aeration because of a coarser texture and a very low C:N ratio.

Muskingum rates are second highest, also due to a combination of factors. The soil texture was second highest in percent sand for better aeration, and it was highest in organic matter content which resulted in the highest control mineralization rate. The pH was slightly less than optimal for most ammonifying organisms, but not as low as the finer textured Brookston (pH 4.8), also high in organic matter but with a slightly wider C:N ratio.

Even though the Hoytville pH was optimum (7.2) for mineralization, poorer aeration due to the finer texture accounts for the lower mineralization rates. Percent organic carbon of the Hoytville is not representative of the organic matter content as significant organic matter not fully decomposed to humus was present.

The Crosby mineralization rates were the lowest, reflecting not only the sub-optimal pH, but also the low soil organic matter present.

The soils highest in organic matter mineralized the most N in the controls with exception of Hoytville. The average N mineralized did not vary substantially in the treated soils for the same temperature.

As expected from the mineralization rate data, Mahoning mineralized the greatest amount of N and had the highest percent N mineralized at both temperatures; Crosby had the least.

Lower than predicted percent N mineralized occurred in the Muskingum soil. Plots of data reveal a more pronounced lag period for this soil at the lower temperature and an early mineralization peak

TABLE 5.9. AVERAGE N MINERALIZATION RATES FOR SOILS BASED ON SLUDGES WHERE N MINERALIZATION RATE EXCEEDED CONTROL N MINERALIZATION RATE

Soil	25 C	15 C	Control
		μg N/g soil/wk$^{1/2}$	
Brookston	52.4	52.3	24.2
Crosby	38.0	45.2	19.1
Hoytville	45.4	50.7	11.5
Mahoning	74.7	63.2	25.3
Muskingum	58.4	57.4	26.4

TABLE 5.10. AVERAGE CUMULATIVE N MINERALIZATION FOR SOILS BASED ON SLUDGES WHERE N MINERALIZATION EXCEEDED CONTROL

Soil	25 C	15 C	Control
		μg N/g soil/8 weeks	
Brookston	116.8	73.9	41.0
Crosby	90.1	62.6	27.5
Hoytville	90.1	77.7	20.9
Mahoning	127.5	83.9	35.2
Muskingum	88.7	69.7	44.3

reached at the higher temperatures. Probable cause for these early peaks may be due to the low soil Mg and/or P content. Because no nutrient solution was used, these may have become limiting factors for mineralization, especially if sludge P was unavailable or immobilized.

Temperature Effect

Differences in temperature (Tables 5.6-5.10) did not contribute significantly to average soil-sludge mineralization rates as they were determined. Comparing the temperature differences of average mineralization rates of the sludges, one finds the rates are similar for both temperatures if the C:N ratio of the sludge was similar (Jackson Pike, Medina 100). The lower Zanesville rate at the lower temperature reflects the higher C:N ratio instead of a temperature effect. Likewise, the higher Defiance rate at 15 C demonstrates the lower C:N ratio. The low Medina 300 rate at 15 C, as stated before, is probably a result of more complex types of organic constituents in the sludge. That mineralization rates were not affected by the 10 C difference in temperature is also evident when one considers the average mineralization rates of the controls.

Tables 5.7, 5.8 and 5.10 show an obvious reduction of the average cumulative N and percent N mineralized at the lower temperature. This is due to a lag period or the extra amount of time initially needed for the more cold tolerant organisms to become established before steady mineralization occurred. These figures at the lower temperature would eventially have equalled those at the higher temperature had the incubation time been extended for the 15 C treatments, due to the similar rates for both temperatures.

pH Effect

Soil pH effect on N mineralization has been mentioned. This discussion will be concerned with the special limed and unlimed Crosby soil and Jackson Pike sludge treatments specifically set up to make a more controlled comparison. This data (Table 5.11) clearly show the mineralization rate, total N above control and percent N mineralized of the limed Crosby (pH 6.6) soil and Jackson Pike sludge is nearly double that of the unlimed treatment (pH 4.7). These results demonstrate a dramatic effect of soil pH when sludge is added. However, this effect is not seen on the untreated soil.

Cadmium Effect

The results of this comparison of Muskingum soil with a high and low cadmium Zanesville sludge vary depending on which data are used (Table 5.11). There is no high/low cadmium effect if one considers the higher set of mineralization figures. As stated before, however, the lower set of figures appears to be the most representative. In this case the mineralization rate of the low Cd sludge is higher than that of the high Cd sludge. This could represent a misplacement of the curve for the low Cd sludge, since total N mineralized is the same for both

TABLE 5.11. EFFECT OF SOIL PH AND SLUDGE CADMIUM CONCENTRATION ON N MINERALIZATION (INCUBATED AT 25 C)

Treatment	N Mineralization Rate	Cumulative N Mineralized	Percent of Sludge Organic N Mineralized
	(μg N/g soil/wk)	(μg N/g soil/8 wks)	
Muskingum	21.9	45.4	---
Muskingum + High Cd Zanesville*	42.4	83.9	31.0
Muskingum + High Cd Zanesville†	11.1	32.0	---
Muskingum + Low Cd Zanesville*	36.2	88.6	28.0
Muskingum + Low Cd Zanesville†	20.5	30.0	---
Unlimed Crosby	15.5	32.7	---
Unlimed Crosby + Jackson Pike	36.1	92.3	21.9
Limed Crosby	11.3	30.9	---
Limed Crosby + Jackson Pike	63.5	145.5	42.0

* Calculations based on one replicate.
† Low = 188 μg/g; High = 544 μg/g.

treatments. In any case, both the rate and amount of mineralization are below those of the control. The relatively high C:N ratio of the Zanesville sludges and thus, increased immobilization is responsible for decreased mineralization. These data suggest the levels of cadmium did not seem to have an effect on N mineralization although sub-optimal experimental controls render inconclusive evidence of this factor.

Nitrification

The percentage of nitrate increased over time while the amount of NH_4^+ leveled off. This shows that proper environmental conditions existed for nitrification in most treatments. Since the microbial population responsible for nitrification in soils (primarily Nitrosomonas and Nitrobacter) represents a much smaller, less diverse group of organisms, they are much more sensitive to less-than-optimum or undesirable conditions than those organisms responsible for mineralization. An acid pH is one of these unfavorable conditions. There was a relatively greater accumulation of NH_4^+ for the Defiance sludge, especially in combination with the Crosby and Brookston low pH soils. That NH_4^+ accumulation for the acid soils was more pronounced with the Defiance sludge illustrates a sludge component contributed to nitrification inhibition.

Nitrification was inhibited in the Zanesville and Muskingum treatments as well. This influence must be solely due to the sludges, since no other Muskingum treatments displayed this dramatic effect. Both the low and high cadmium sludges affected nitrification equally, which eliminates cadmium as the inhibitor.

Substantial NH_4^+ accumulated through the eighth week at 15 C while significant NH_4^+ accumulation ceased after the second week at 25 C. These results demonstrate 25 C is more optimal than 15 C for nitrifying bacteria.

SUMMARY AND CONCLUSIONS

Nitrogen mineralization of sludge in soils is extremely variable as a result of differing soil, sludge and environmental factors. The wider the sludge C:N ratio, the lower the rate, cumulative, and percent N mineralized. Degree of sludge treatment influences mineralization in the following manner. The aerobically digested sludges (Medina) have been more thoroughly degraded and are therefore more stable as evidenced by their narrow C:N ratio. This is reflected in a generally high mineralization rate but an early mineralization peak resulting in less cumulative N and percent N mineralized. The latter indicates relative resistance of the remaining organic material to further decomposition. The more select group of organisms required to degrade these more complex sludge constituents appeared to be more affected by the cooler temperature, displayed in a longer lag period.

Three major soil factors influencing net N mineralization are pH, texture or internal degree of aeration and C:N ratio. When near optimal levels of each are present, as in the Mahoning soil, the consequence is synergistically increased mineralization. With only one component suboptimal, as in the heavier textured Hoytville, the average percent N mineralization was quite high while the rate and total N mineralized was relatively low. Net N mineralization in this treated soil above the control is exceptionally high, perhaps due to a sludge stimulation. The extreme soil pH effect on N mineralization is rather surprising when one considers the great numbers and diversity of organisms responsible for ammonification.

That the 10 C temperature difference had little effect on mineralization rate is unexpected. More than likely the manner in which the rate was determined here, with lag points and points past the mineralization peak disregarded, is responsible for this. There is no question total N and percent N mineralized were greater at the higher temperature. Average figures for the latter are 22.4 percent at 15 C and 30.2 percent at 25 C. This consistence with reported values is gratifying.

Cadmium effect on N mineralization cannot be determined by the data presented here. With wide sludge C:N ratios, little net N mineralization occurred as a result of increased immobilization.

In most of the treatments nitrification occurred. As one would suspect, a pH less than 5.0 slowed nitrification. Ammonium continued to accumulate the seventh and eighth week at the cooler temperature, while at 25 C quantities ceased to increase significantly after the second or third week. Defiance and Zanesville sludges showed evidence that a nitrification inhibitor was present.

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SECTION 6

FACTORS AFFECTING AMMONIA VOLATILIZATION FROM SEWAGE SLUDGE APPLIED TO SOIL IN A LABORATORY STUDY

William C. Donovan, B.S., M.S., Ph.D.
Terry J. Logan, B.S., M.S., Ph.D.
The Ohio State University
Columbus, Ohio 43210

INTRODUCTION

There has been an increasing use of municipal sewage sludge on agricultural land in the U.S. in the last decade. States like Ohio (Miller et al., 1979) recommend application rates that will supply part or all of the nitrogen and/or phosphorus needs of the crop. About 50% of the total nitrogen in digested sewage sludge is organic-N and the remaining 50% is ammonia (Sommers, 1977). Digested sludges contain only trace amounts of NO_3-N. The nitrogen available to a crop will include the mineralizable fraction of organic-N and all of the ammonia. However, some of the ammonia fraction will be susceptible to volatilization losses; and the extent of this loss must be determined if the farmer is to have an accurate estimate of the nitrogen supplied to his crop by sludge. Reliable estimates of plant-available nutrients in sludges is particularly important when the sludge is being sold for its nutrient value.

Although there have been few direct measurements of volatilization losses of NH_3 from sewage sludges (Beauchamp et al., 1978), the literature is replete with studies on losses from ammonia fertilizers (Ernst and Massey, 1960; Gasser, 1964; Martin and Chapman, 1951; Volk, 1961) and livestock wastes (Hoff et al., 1981). These studies show that, among other factors, ammonia volatilization is affected by: wind speed, time of incorporation, pH, temperature, soil moisture, soil cation exchange capacity and base saturation, and vegetative cover.

The objective of this study was to determine the relative effects of several of these factors and the effects of sludge type on NH_3 volatilization from sewage sludges applied to soil in a laboratory study.

METHODS AND MATERIALS

Ammonia Collection System

The ammonia collection system used for this study was based upon equipment designed by Kissel et al. (1977) as modified by Hoff et al. (1981). The system (Figure 6.1) consisted of four parts: (1) an acid scrubber to remove ambient ammonia in the incoming air; (2) a volatilization cylinder enclosing the sludge-treated soil to sample the air above the soil surface; (3) an acid trap to retain the ammonia volatilized from the sludge; and (4) a vacuum pump to pull air through the system. The system also contained two manifolds to conduct air from the scrubber through the volatilization cylinder and into the acid trap.

The scrubber consisted of a 500-ml narrow-mouth polyethylene bottle containing 300 ml of 0.5N H_2SO_4. A dispersion tube bubbled air through the scrubber before it was drawn into the volatilization cylinder.

The volatilization chamber consisted of a 30-cm diameter PVC cylinder cut to a length of 25 cm. The cylinder was inserted to a depth of 21 cm in the soil so that the upper 4 cm of the cylinder was above the soil surface. Five 5-cm (internal diameter) air inlet tubes were evenly spaced around one-half of the circumference of the cylinder with one outlet directly across from the inlets to pull air across the soil surface during sampling. The inlets and outlets were 2 cm above the soil surface.

The cylinder was placed inside a polyethelene bin measuring 47 cm by 35 cm on the sides by 18 cm deep. The volatilization cylinder was closed during sampling with a pyrex glass lid sealed around the edges with vacuum grease.

The NH_3 trap consisted of two 2.5-liter glass bottles connected in series. Each bottle contained 800 ml of 2% boric acid. The second bottle was present to slow the air flow and to provide additional capacity to trap NH_3.

A vacuum pump with a free air displacement of 760 liters/minute pulled air through the system. The manifold system enabled three volatilization cylinders to be sampled at one time with an air exchange of 18 volumes per minute per cylinder.

The ammonia collection system was calibrated by mixing solutions of known ammonium chloride concentration with 0.5N NaOH. Acid was added later to stop the generation of NH_3. The efficiency of the system was determined by using semi-micro Kjeldahl analysis to determine both NH_3 remaining in the solution and NH_3 trapped in the boric acid. NH_3-N recovery was determined for different amounts of ammonia generated and at different air flow rates. Recoveries ranged from 80-120% with the

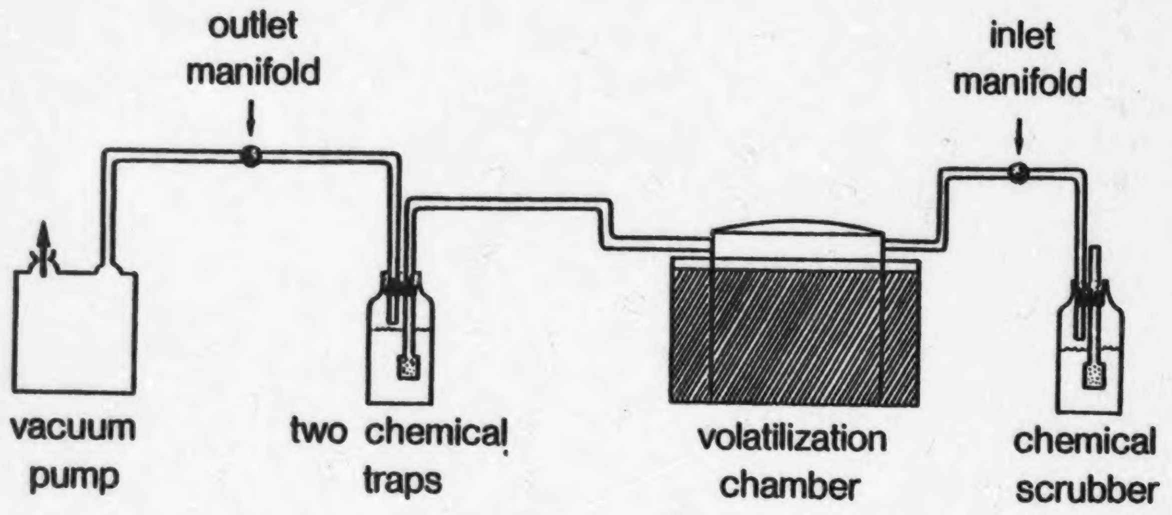
outlet manifold
inlet manifold
vacuum pump
two chemical traps
volatilization chamber
chemical scrubber

Figure 6.1 Side view of the volatilization apparatus (not drawn to scale).

highest NH_3-N recovered at the lowest air flow and for the lowest NH_3-N generated. The lowest air flow rate (18 air exchanges/minute) was used for all experiments, and NH_3-N recovered in the experiments did not exceed the lowest NH_3-N generated during calibration. It was, therefore, assumed that recovery during the experiments was approximately 90-100% and the data were not corrected to 100% recovery.

The air flow rate (18 air exchanges/minute) during sampling corresponded to a wind speed of 0.22 km/hr. and was greater than rates that were found by Kissel et al. (1977) and Fenn and Kissel (1973) to produce maximum volatilization. During non-sampling periods, wind speed in the laboratory was calculated to be 0.11 km/hr., which is still close to the air speed for maximum volatilization found by Kissel et al. (1977) and Fenn and Kissel (1973). The objective of this research, however, was to determine relative effects on NH_3 volatilization and not absolute NH_3 losses.

Preparation of Soil and Sludge Samples

The Crosby silt loam (fine, mixed, mesic Aeric Ochraqualf) used in this study was taken from experimental field plots. Soil for all experiments except pH was collected from a plot with a pH of 6.7. Soil was collected from two other plots with a pH of 5.1 and 7.5, respectively, for the pH experiment. The soils were air-dried and screened (<2 mm) prior to use.

The soil was moistened to the desired water content prior to being placed in the bin containing the PVC cylinder. The soil was then placed both inside and outside the cylinder and firmed. Additional soil was added and firmed until the soil surface was 4 cm below the rim of the volatilization cylinder. The bin, cylinder, and soil were then immediately covered with plastic to prevent moisture loss until the experiment began.

The soil in each bin was totally replaced between each experiment, and the top 11 cm of soil in each bin and volatilization cylinder was replaced between each run.

Samples of dewatered, anaerobically digested, sludge were collected at the Jackson Pike sewage treatment plant in Columbus, Ohio. This material was used for all of the experiments. In addition, for the experiment with different sludges, samples were collected at treatment plants in Medina and Ashland, Ohio, stored in plastic containers, and sent to Columbus. The compost sample was collected from the composting facility at the Columbus, Ohio Southerly treatment plant. All sludge samples were stored at 1.1 C until used.

For all of the experiments except sludge type, the dewatered Columbus sludge (17-28% solids) was diluted to 10% solids and applied at a rate of 5 Mg ha^{-1} (dry weight).

For the experiment with different sludge types, the application rate was 2.5 Mg/ha (dry weight). The solids content was 5% for the Columbus anaerobically digested liquid sludge, 1.57% for the Medina aerobically digested sludge, 1.96% for the Ashland lime-stabilized primary sludge, 61.2% for the composted Columbus primary sludge, and 17.3% for the dewatered Columbus sludge. The ammonia content, NH_3-N applied per cylinder and sludge volume applied per cylinder are given in Table 6.1 for the experiment with different sludges.

Half of the sludge was applied to the soil surface outside the PVC cylinder and half inside, as the area inside the cylinder was 50% of the total surface area of the bin. The sludge was sampled for NH_3 analysis just prior to its application to the soil.

The sludge was prepared within 15 minutes of the start of the experiment. The plastic covering the soil was removed, and the sludge applied. The individual cylinders were closed with pyrex glass lids and the pump turned on. The sampling period was 20 minutes and began when the trap started bubbling. Samples were taken at 0, 1, 3, 6, 12, and 24 hours after application of the sludge. The cylinders were uncovered between sampling periods.

Ammonia Analysis

Ammonia recovered in boric acid was determined by titration with dilute acid. Samples were titrated within 1 hour after their collection. The lower detection limit was 120 µg NH_3-N. NH_3-N in the liquid sludges was determined by steam distillation with MgO. NH_3-N in the compost was determined by extraction with 2N KCl (10:1 ratio by weight) and subsequent steam distillation.

Statistical Analysis

The Statistical Analysis System (SAS) package at The Ohio State University Instruction and Research Computer Center was used for data analysis. The best fit line of NH_3-N volatilized versus time was determined by regression analysis, and the integrated form of the best-fit line was used to calculate NH_3 volatilized for any period up to 24 hours. Distinctness or separateness of volatilization curves of individual treatments was determined at the 0.05 level according to Neter and Wasserman (1974). The calculated values of NH_3-N volatilized in the 24 hr. sampling period were expressed as percent of total NH_3-N applied. Analysis of variance and Duncan's Multiple Range Test was used to determine differences between treatment means.

TABLE 6.1. SLUDGE TREATMENT AND NH_3-N AND SOLIDS CONTENT OF THE SEWAGE SLUDGES STUDIED IN EXPERIMENT 4

Sludge Name	Sludge Treatment	NH_3-N Content (µg/g dry solids)	Solids Content %	Amount Applied Per Cylinder for a 2.5 Mg ha^{-1} Application: NH_3-N (mg)	Volume (ml)
Ashland	Lime-stabilized liquid primary sludge, pH 12	11 400	2.0	205	900
Medina	Aerobically digested liquid sludge	7 400	1.6	133	1 120
Columbus	Anaerobically digested liquid sludge	8 100	5.0	142	350
Dewatered Columbus	Anaerobically digested, centrifuged sludge	8 300	17.3	147	102
Columbus Compost	Composted primary sludge	900	61.2	17	30

Experimental Design

A series of experiments were run in which all treatment variables were kept constant except the variable being studied. Experiments included: soil moisture, time of incorporation, soil pH, sludge type, temperature and vegetative cover. The treatments are summarized in Table 6.2.

The experimental treatments were replicated from 3-18 times, depending on the number of treatments in an individual experiment. Since a total of six cylinders could be run at one time, the number of runs which were required to give the necessary replications varied with each experiment. In each experiment, however, all of the treatments were included in each run and were randomized as to their positions on the experimental apparatus.

The soil was incorporated to a depth of 3.5 cm for Experiment 2 (Time of Incorporation).

Experiment 5 (Temperature) was run in a growth chamber. Temperature was recorded throughout the experimental period and the range and means for the three runs were: 12-14.5, 12.8 C; 17.8-18.9, 18.3 C; 24.5-28.9, 26.7 C.

For Experiment 6 (Vegetative Cover), the soil inside and outside the cylinders that were to contain straw and sod was removed until the soil level was 6 cm below the rim of the PVC cylinders. The grass sod or straw placed inside the cylinders raised the level to 4 cm from the rim of the cylinders, the standard condition in the other experiments.

The experiment was divided into two parts: one with sludge containing large sludge particles (~ 1 mm or greater) and another with well-homogenized sludge (without large sludge particles).

A second batch of Columbus sludge had to be collected since the original supply (at 17.3% solids) had been depleted in previous experiments. This newly collected sludge had a solids content of 27.7% and was mixed with distilled water to give a solids content of 10%.

A total of four runs were made with two replications per treatment in each run for a total of eight replications per treatment. It was observed during these runs that the second batch of sludge contained large sludge particles which were retained by the sod and straw. To evaluate this effect, the sludge was carefully homogenized after dilution to 10% solids and a new series of three runs were made with two replications of each treatment per run for a total of six replications per treatment.

TABLE 6.2. A SUMMARY OF THE NH_3 VOLATILIZATION EXPERIMENTS

Experiment	Reference Conditions[*]	Variable Conditions	Replications
1. Soil Moisture[†]	0.01 MPa	0, 1.5 MPa Air-dry (3.1 MPa)	6 12
2. Time of Incorporation	Unincorporated[‡]	0.25, 1, 3, 6, 12 hours after application	5
3. Soil pH	6.7	5.1, 7.5	6
4. Sludge Type	Columbus anaerobically digested, liquid	Ashland lime-stabilized primary Medina aerobically digested Columbus anaerobically digested, dewatered Columbus composted primary	3
5. Temperature	26.7 C[§]	12.8, 18.3 C	6
6. Vegetative Cover			
a) Large sludge particles	bare	Wheat straw Kentucky bluegrass sod	8
b) Homogenized sludge	bare	Wheat straw Kentucky bluegrass sod	6

[*] These conditions were kept constant in each experiment except for the variable (e.g., temperature) being studied.

[†] The moisture contents at 0, 0.01, 1.5 and 3.1 MPa (megapascals) were 32, 22, 10 and 5.9% by weight, respectively.

[‡] In this experiment, sludge was incorporated just before the 24-hour sampling.

[§] In the laboratory for the other experiments, temperature was 26 ± 2 C.

RESULTS AND DISCUSSION

Experiment 1: Soil Moisture

NH_3-N volatilized per cylinder is shown graphically in Figure 6.2. The greatest ammonia loss occurred from sludge applied to the soil at 0 MPa tension (saturation; 32% moisture). Peak ammonia loss occurred at one hour and decreased gradually throughout the experimental period. At all times, ammonia losses were higher than at other moisture contents. The 1.5 MPa treatment was second in terms of ammonia loss throughout the experimental period, followed closely by the 0.01 MPa treatment. The air-dry soil gave the lowest NH_3 loss at each sampling period.

The pattern of volatilization for the air-dry treatment was statistically significant from the other three treatments, but the pattern of volatilization for the 0, 0.01 and 1.5 MPa treatments were not different from each other (Table 6.3). Four to five times less NH_3 loss occurred in the 24-hour sampling period from the air-dry soil than from soil at higher moisture contents (Table 6.3).

The additional water added to the soil with the sludge (10% solids; 90% H_2O) increased soil moisture: for the air-dried soil (3.1 MPa), soil moisture increased from 6 to 8.5%; for the 1.5 MPa soil, from 10 to 12.8%; for the soil at 0.01 MPa, soil moisture increased from 22 to 24.2%; and for the saturated soil, soil moisture increased from 32 to 34.5%, assuming that the sludge liquid interacted with the entire volume of soil in the bin. This lowered the moisture tensions for the 0.01, 1.5 and 3.1 MPa treatments to 0.007, 1.05 and 1.89 MPa, respectively. The 0 and 0.01 MPa treatments were essentially the same after sludge was applied, but the increased H_2O content after sludge application does not explain why the 1.5 MPa treatment had the same NH_3 loss as the more water-saturated soils. The literature has shown that there are several competing mechanisms which determine the effects of soil moisture on NH_3 volatilization. In the case of the 0 and 0.01 MPa treatments, there was a free liquid surface (ponding) during most of the 24 hour sampling period, and Wahhab et al. (1957) and others have shown that evaporation of water is important in NH_3 volatilization. With these two treatments, also, there was a minimum of contact between the sludge liquid containing the dissolved NH_3 and the soil. This reduced the ability of the soil to absorb and hold ammonia.

In the case of the 1.5 MPa and air-dry treatments, moisture tensions were high enough after addition of the sludge to absorb the sludge liquid. The only difference noted between these two treatments was the observation that the air-dry soil (3.1 MPa) absorbed the liquid sludge more rapidly and to a greater depth than the 1.5 MPa soil. Absorption of the sludge liquid to a shallow depth would have resulted in a much higher moisture content and lower moisture retention than was calculated for the entire volume of soil in the pan. Also, movement of

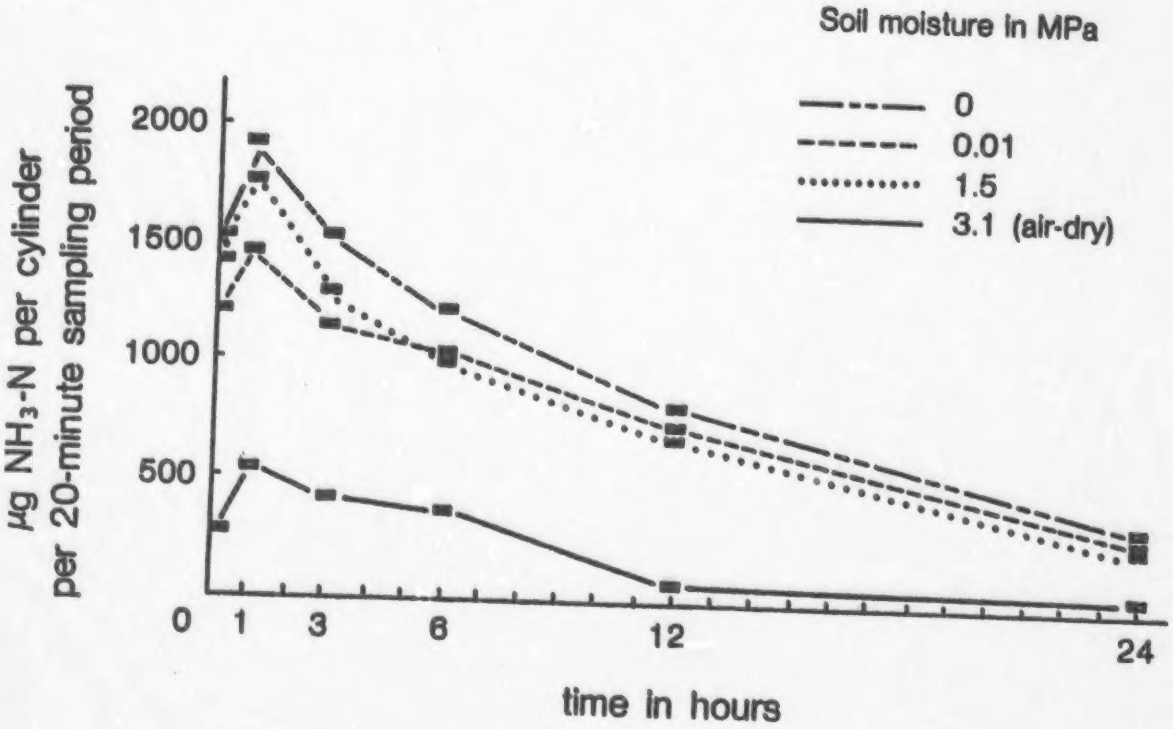

Figure 5.2 NH_3-N volatilized versus sampling period for sewage sludge applied to soils at 0, 0.01, and 1.5 MPa, and air-dry initial moisture levels. Horizontal bars indicate the 20 minute sampling period.

TABLE 6.3. NH_3-N VOLATILIZED AS PERCENT OF NH_3-N APPLIED AND TESTS OF SIGNIFICANCE FOR SEWAGE SLUDGE APPLIED TO SOILS AT 0, 0.01 AND 1.5 MPA, AND 3.1 MPA (AIR-DRY) INITIAL MOISTURE LEVELS

Treatment MPa	NH_3-N Volatilized in 24 Hours as Percent of NH_3-N Applied	Test of Line Equality*	Duncan's Multiple Range Test*
0	31.6	a	a
0.01	25.9	a	a
1.5	26.8	a	a
3.1 (air-dry)	6.4	b	b

*Means followed by the same letter are not significantly different at the 0.05 level.

the sludge liquid containing NH_3 to a greater depth in the air-dry soil increased the depth of soil through which NH_3 gas would have to diffuse to the surface.

A statistically significant increase in the 24-hour cumulative NH_3-N volatilization per cylinder with increasing initial soil moisture was given by regression equations in linear, quadratic, and cubic forms, respectively:

	r^2	p
$\%NH_3\text{-}N = 6.64 + 0.86w$	0.37	0.0003
$\%NH_3\text{-}N = -4.86 + 2.75w - 0.05w^2$	0.42	0.0006
$\%NH_3\text{-}N = -58.99 + 15.41w - 0.05w^2 + 0.01w^3$	0.55	0.0001

where w = percent soil moisture

Experiment 2: Sludge Incorporation

NH_3-N volatilization decreased when sludge was incorporated (Figure 6.3). The greatest reductions occurred when sludge was incorporated immediately. Table 6.4 indicates that the pattern of volatilization for the sludge incorporated immediately was distinct from those of the other periods of incorporation. Percent NH_3-N volatilization for the sludges incorporated 0.25-12 hours after application were statistically lower than the NH_3 loss from sludge incorporated at 24 hours (Table 6.4). The four to eight fold reduction in volatilization when sludge was incorporated within three hours after application (Table 6.5) versus the 24-hour incorporation indicates that significant nitrogen conservation can be achieved through timely incorporation of the sludge after its application.

There was a linear increase ($p = 0.0001$), $r^2 = 0.43$, in volatilization with time before incorporation:

$\%$ NH_3-N volatilized/cylinder = 3.62 + 0.87 (time of incorporation, hours)

For incorporation at 1, 6, and 24 hours, the calculated values for percent NH_3-N volatilized are 4.5, 8.8, and 24.5%, respectively. This compares to actual values of 4.0, 8.8, and 25.8%, respectively.

Experiment 3: Soil pH

Increasing soil pH increased ammonia volatilization (Table 6.5). The general shapes of the curves (not shown) however, were quite similar, and were not distinct (Table 6.5). The volatilization loss at pH 7.5 was significantly higher than the losses at the lower pH's.

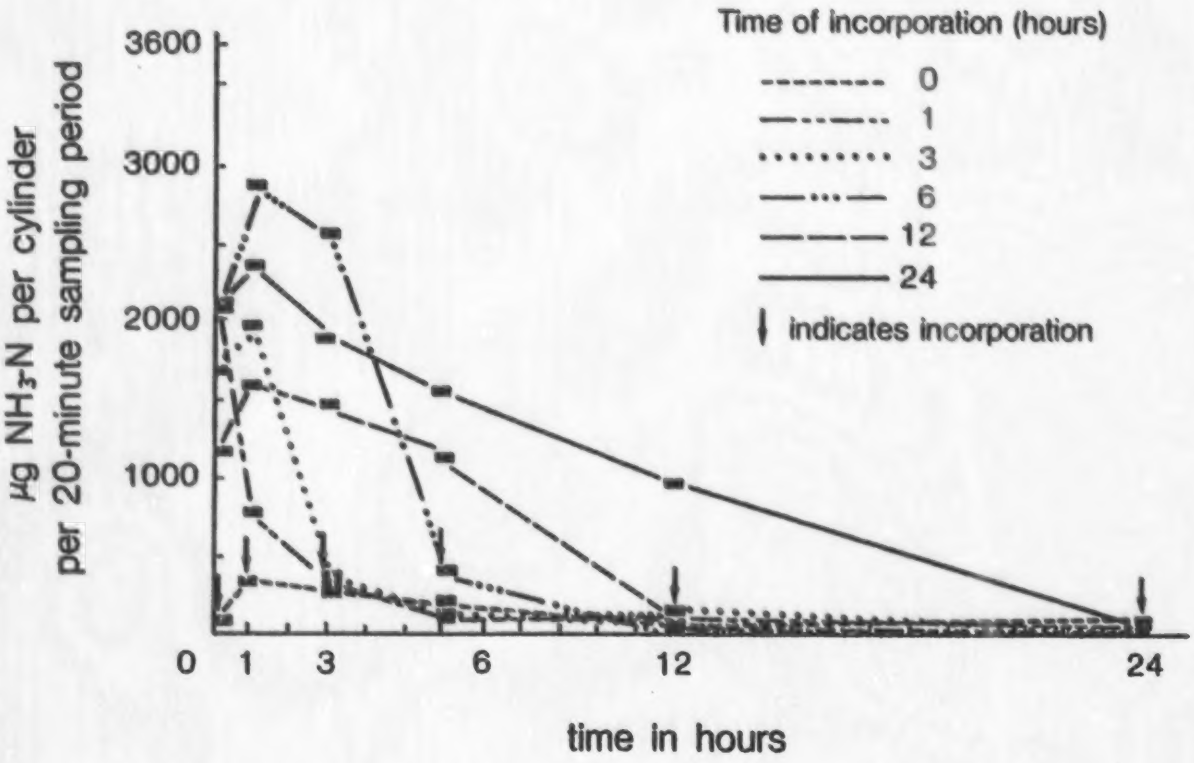

Figure 6.3. NH_3-N volatilized versus sampling period for sludge incorporated 0.25, 1, 3, 6, 12, and 24 hours after application.

TABLE 6.4. NH_3-N VOLATILIZED AS PERCENT OF NH_3-N APPLIED AND TESTS OF SIGNIFICANCE FOR SEWAGE SLUDGE INCORPORATED IN SOIL AT 0.25, 1, 3, 6, 12, AND 24 HOURS AFTER APPLICATION

Periods of Incorporation (hours)	NH_3 Volatilized Over 24 Hours as % of Total NH_3-N Applied	Test of Line Equality*	Duncan's Multiple Range Test*
0.25	3.1	a	a
1	4.0	b	a
3	6.3	b	a
6	14.2	bc	ab
12	13.6	bc	ab
24	25.8	c	b

*Means followed by the same letter are not significantly different at the 0.05 level.

TABLE 6.5. NH_3-N VOLATILIZED AS PERCENT OF NH_3-N APPLIED AND TESTS OF SIGNIFICANCE FOR SEWAGE SLUDGE APPLIED TO SOIL PH'S OF 5.1, 6.7, AND 7.5

Soil pH	NH_3-N Volatilized Over 24 Hours as % of Total NH_3-N Applied	Test of Line Equality*	Duncan's Multiple Range Test*
5.1	20.0	a	a
6.7	21.0	a	a
7.5	23.6	a	b

*Means followed by the same letter are not significantly different at the 0.05 level.

There was a statistically significant increase in the 24-hour cumulative NH_3 loss per cylinder with increasing soil pH:

	r^2	P
$\%NH_3\text{-}N = 2.65 + 2.12\ pH$	0.30	0.028
$\%NH_3\text{-}N = 90.94 - 26.68\ pH + 2.30\ (pH)^2$	0.45	0.022

The linear equation gave a better fit between predicted and observed data (not shown).

	r^2	P
$\%NH_3\text{-}N = 2.65 + 2.12\ pH$	0.30	0.028
$\%NH_3\text{-}N = 90.94 - 26.68\ pH + 2.30\ (pH)^2$	0.45	0.022

The linear equation gave a better fit between predicted and observed data (not shown).

Previous work (Ivanov, 1964) has shown that the greatest effect of soil pH on ammonia volatilization occurs at high pH's, and particularly when the soil is calcareous. The pH 7.5 Crosby soil used in this study did not have free carbonates. One other factor may have reduced the effect of soil pH on NH_3 loss. The soil in this experiment had an initial moisture content of 0.01 MPa, which has been previously shown to reduce the contact between the sludge liquid and the soil. This would reduce the ability of the soil to change the solution pH which was 7.2 for the sludge itself.

Experiment 4: Sludge Type

There were large differences in the ammonia volatilized from the different sludges (Figure 6.4 and Table 6.6), but some of these differences are because different amounts of NH_3-N were applied for the different sludges (Table 6.1). Approximately 1.5 times as much NH_3-N was applied with the Ashland lime-stabilized liquid primary sludge as for the Medina aerobically digested sludge, Columbus, and dewatered Columbus anaerobically digested sludges. Only 0.12 times as much NH_3-N was applied with the Columbus compost as with the Medina, and Columbus and dewatered Columbus sludges.

The Ashland sludge (Figure 6.4) had very high NH_3-N losses throughout the 24 hour period. The ammonia values dropped off rapidly for the six hour sampling and then more gradually over the remainder of the sampling period. The pH of the Ashland sludge, a lime-stabilized sludge, is 12, and this was responsible for the rapid loss of ammonia. Table 6.6 shows that 15.8% of the ammonia was volatilized in 24 hours. The percentage NH_3-N losses in this experiment should not be compared to the results from the other experiments as the sludges were applied at 2.5 Mg ha^{-1} compared to 5 Mg ha^{-1} for the other experiments.

The pattern of ammonia volatilization for the different sludges fell into three groups: Ashland; the two Columbus sludges; and the

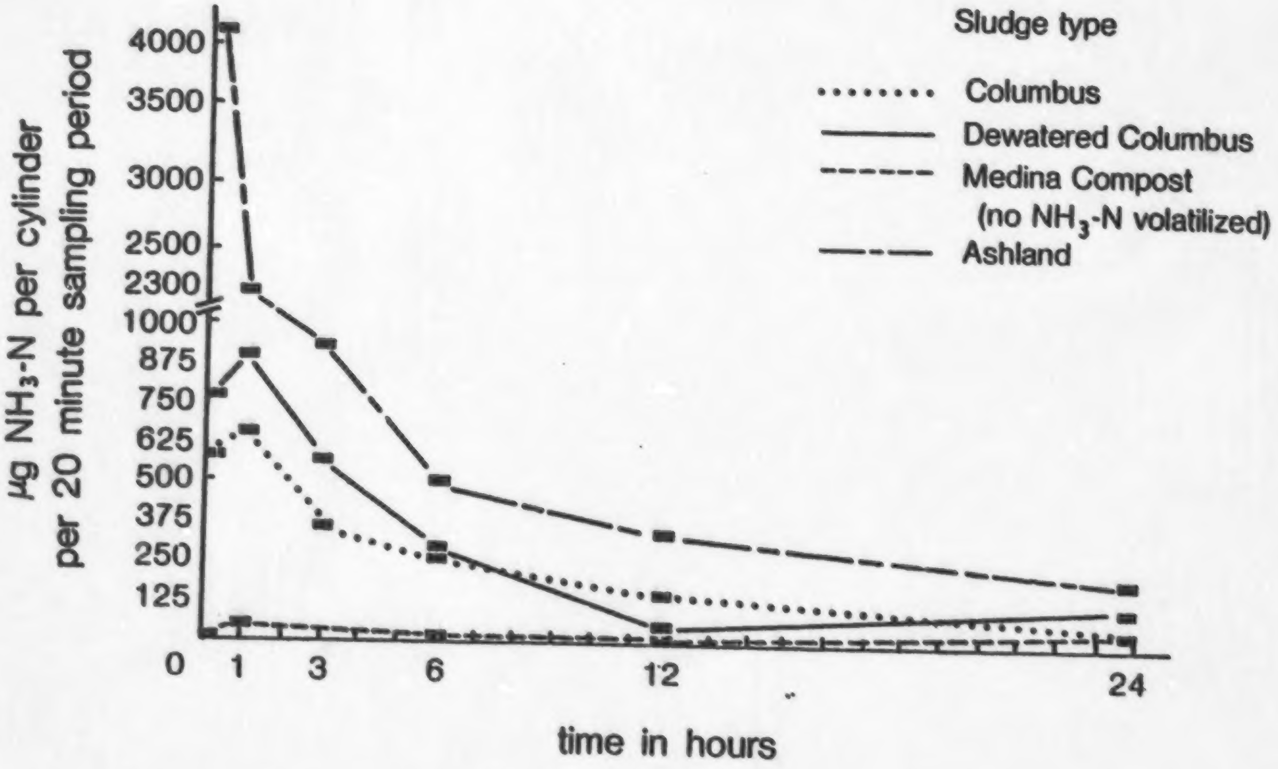

Figure 6.4. NH_3-N volatilized versus sampling period for an Ashland primary lime-stabilized sludge, a Columbus anaerobically digested sludge, a composted Columbus primary sludge, a Medina aerobically digested sludge, and a dewatered Columbus anaerobically digested sludge applied to soil.

TABLE 6.6. NH_3-N VOLATILIZED AS PERCENT OF NH_3-N APPLIED AND TESTS OF SIGNIFICANCE FOR A COLUMBUS ANAEROBICALLY DIGESTED SLUDGE, A DEWATERED COLUMBUS ANAEROBICALLY DIGESTED SLUDGE, A MEDINA AEROBICALLY DIGESTED SLUDGE, A COMPOSTED COLUMBUS PRIMARY SLUDGE, AND AN ASHLAND LIME-STABILIZED PRIMARY SLUDGE

Sludges	Type of Sludge	NH_3 Volatilized Over 24 Hours as % of Total NH_3-N Applied	Test of Line Equality*	Duncan's Multiple Range Test*
Columbus	anaerobically digested	8.3	a	a
Dewatered Columbus	anaerobically digested	7.8	a	a
Medina	aerobically digested	0.4	a	a
Columbus Compost	primary	none detected	a	a
Ashland	aerobically digested, lime-stabilized	15.8	b	b

*Means followed by the same letter are not significantly different at the 0.05 level.

Medina sludge and Columbus compost. However, only the Ashland sludge gave a pattern of volatilization that was statistically significant from the others (Table 6.6). The Ashland sludge had significantly more NH_3 loss in the 24 hour sampling period than did the other sludges. The lack of statistical significance in NH_3 loss among the other sludges was due, in part, to the lower number of replications per treatment (three) in this experiment and to the very high volatilization of the Ashland sludge compared to the others. The number of replications for this experiment was limited by the number of treatments and the amounts of the different sludges available.

Of the different sludges studied, the lime-stabilized material is the least common for land application in Ohio and other areas. Although it is a good source of phosphorus and nitrogen, these results indicate that much of the NH_3 (which usually accounts for 60-70% of the available N in municipal sewage sludges) can be lost if not immediately incorporated. Other problems with lime-stabilized sludge such as potential odor and physical handling reduce the suitability of this material for application to cropland.

Experiment 5: Temperature

The pattern of volatilization for the 18.3 and 26.7 C temperatures (not shown) were similar, with the ammonia volatilization values greater for the 26.7 C temperature compared to the 18.3 C temperature. The principal effect seems to be at the first sampling, where volatilization at 18.3 C was approximately 2/3 that at 26.7 C. At the one-hour sampling, the respective values were closer, with the 18.3 C temperature value being 80% of the NH_3-N volatilization at 26.7 C. The patterns of volatilization were distinct from each other (Table 6.7) and from the 12.8 C treatment. The percent of applied NH_3-N volatilized in 24 hours from the 26.7 C temperature was 13.6% compared to 9.8% from the 18.3 C temperature (Table 6.7), but these differences were not statistically significant.

The pattern of volatilization for the 12.8 C temperature showed much lower volatilization and declined much more gradually than at the two higher temperatures. For the 24 hour period, 2.3% of the applied ammonia was volatilized (Table 6.7), which was significantly lower than the other two temperatures.

Regression analysis showed that volatilization increased with increasing temperature according to the equation ($p = 0.0009$; $r^2 = 0.63$):

$$\%\ NH_3\text{-N volatilized/cylinder} = -28.88 + 3.31\ (C) - 0.07\ (C)^2$$

TABLE 6.7. NH_3-N VOLATILIZED AS PERCENT OF NH_3-N APPLIED AND TESTS OF SIGNIFICANCE FOR SEWAGE SLUDGE APPLIED TO SOILS AT TEMPERATURES OF 12.8, 18.3, AND 26.7 C

Temperature (C)	NH_3 Volatilized Over 24 Hours as % of Total NH_3-N Applied	Test of Line Equality*	Duncan's Multiple Range Test*
12.8	2.3	a	a
18.3	9.8	b	b
26.7	13.6	c	b

*Means followed by the same letter are not significantly different at the 0.05 level.

For temperatures of 12.8, 18.3, and 26.7 C, the predicted percent NH_3-N volatilized was 2.9, 9.9, and 13.1%, respectively. The actual values were 2.3, 9.8, and 13.6%, respectively.

It is common to spread sludge year-round since many sewage treatment plants do not have sufficient storage capacity to avoid spreading for more than a month or so. Sludge spread during the winter is not incorporated because of frozen soil, but these data would indicate that NH_3 volatilization losses would be quite low during these periods. On the other hand, much sludge is spread in the summer months, particularly on fields from which wheat has been harvested and on hay and pasture lands. Surface temperatures during this period can greatly exceed the maximum temperature of 26.7 C studied here, and volatilization losses would be expected to be much greater than the 13.6% obtained for that temperature.

Experiment 6: Vegetative Cover (Large Sludge Particles)

A new batch of the Columbus dewatered sludge with a solids content of 27.7% was collected just prior to this experiment and proved difficult to thoroughly mix with distilled water. Small chunks of sludge remained even after thorough mixing. The cover had two forms: wheat straw and a Kentucky blue grass sod cut to a height of 3.5 cm.

The peak of NH_3 loss for both the straw and sod was greater and of longer duration than for the bare soil (Figure 6.5). The straw and sod seemed to reach a plateau in ammonia volatilization from the one hour to the three hour reading, and then declined over time, while the bare soil had a peak reading at one hour and then declined over time. The volatilization pattern for the two vegetative cover treatments were statistically distinct (Table 6.8) from that of the bare soil but not from each other. Volatilization from the bare soil was statistically significant (6.4% of the NH_3-N applied) compared to volatilization from the soil with the wheat straw cover (14.3%), but the sod treatment was not statistically different from the other two (Table 6.8).

When sewage sludge was surface applied to the straw-covered soil, the straw retained some of the sewage sludge chunks preventing them from making contact with the soil. The straw acted as a physical barrier. Meyer et al. (1961) found that, when a urea-ammonium nitrate fertilizer solution was sprayed on a straw residue covering an acid soil, NH_3 loss was similar to that from an alkaline or neutral soil because the straw physically intercepted the solution and volatilization occurred from the spray on the straw surface and not from the soil surface. Something similar appears to have occurred here. The straw had the largest percent ammonia volatilization loss and the grass was next. An additional factor besides the interception of part of the sewage sludge was that the grass sod was very thick, and possibly the air circulation through and around the grass sod was not as efficient as through the

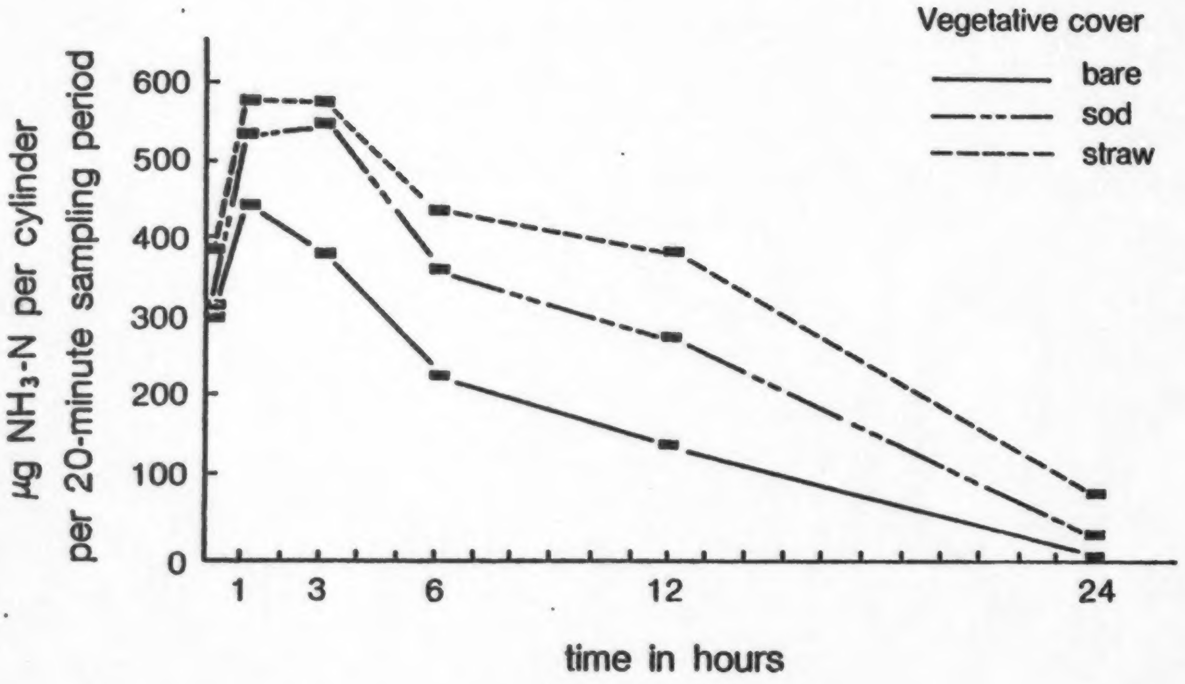

Figure 6.5. NH_3-N volatilized versus sampling period for sewage sludge containing large sludge particles applied to a straw-covered soil, a sod, or bare soil.

TABLE 6.8. NH_3-N VOLATILIZED AS PERCENT OF NH_3-N APPLIED AND TESTS OF SIGNIFICANCE FOR SEWAGE SLUDGE APPLIED TO SOILS WITH VEGETATIVE COVER (WHEAT STRAW OR SOD) AND A BARE SOIL

Vegetative Cover	NH_3-N Volatilized Over 24 Hours as % of Total NH_3-N Applied	Test of Line Equality*	Duncan's Multiple Range Test*
	Large Sludge Particles		
Wheat Straw	14.3	a	a
Sod	11.3	a	ab
Bare Soil	6.4	b	b
	Homogenized Sludge		
Wheat Straw	9.1	a	a
Sod	0.1	a	a
Bare Soil	6.4	a	a

*Means followed by the same letter are not significantly different at the 0.05 level.

straw, allowing more volatilization to occur from the straw. The grass was growing in a vertical direction, while the straw was more horizontal, allowing more of the sludge and water mixture to be physically intercepted by the straw compared to the grass, thus presenting a larger volatilization surface from the straw. The sod was clipped just before the experiment began, and it is possible that the grass took up some of the sludge ammonia, but active growth was not observed until after the experiment was terminated.

Ammonia loss was least from the bare soil, and evidently the contact of the sewage sludge mixture with the soil prevented part of the loss.

Experiment 6: Surface Cover (Liquid Sludge)

The experiment was repeated as described for the first part of Experiment 6. The sludge was mixed with distilled water, allowed to stand for five minutes, then again mixed. The repeated mixing process was sufficient to eliminate the large sludge particles.

Volatilization patterns for the three treatments (not shown) were not distinct (Table 6.8) nor were there any statistically significant differences in the amounts of NH_3 volatilized in the 24-hour experimental period.

The wheat and sod delayed the peak periods of NH_3 volatilization compared to the bare soil as they did in the first part of the experiment with sludge containing sludge particles. The main difference, however, was greater overall volatilization with the vegetation treatments in the first part of the experiment (large sludge particles) but little or no effect of these treatments with the more homogenized sludge. Because of its high water solubility, almost all of the ammonia in liquid sludge is associated with the liquid fraction and little is on the sludge solids. If sludge solids were trapped on the surface of the straw and sod, volatilization of NH_3 from the particles themselves would probably not be enough to account for the differences observed. Another possible explanation, however, is that the solids may have prevented the sludge liquid from rapidly moving through the vegetative cover to the soil surface by plugging the spaces between the straw particles or blades of grass. In addition, the sludge solids are organic and would have retained a high percentage of moisture even after most of the sludge liquid had moved through the vegetative layer and into the soil. Ammonia could have volatilized from the absorbed water.

Although the effects of vegetative cover on NH_3 volatilization from sewage sludges were found to be small, and affected by the physical characteristics of the sludge itself, these effects could be important in the field. Many sludges are land-applied as filter cake or centrifugal sludge which contain around 10-25% solids, and the sludge particle effects noted in this research could be more significant with sludges of this type. Also, application of sludges to land with vegetative cover (wheat stubble, corn stalk residues, hay or pasture land) is a recommended practice wherever feasible to reduce soil compaction and rutting by the applicator truck, and to minimize runoff.

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SECTION 7

SOIL DEGRADATION AND PLANT ABSORPTION OF PCB FROM PCB AMENDED SEWAGE SLUDGE

Iraida Robledo, B.S., M.S.
Robert H. Miller, B.S., M.S., Ph.D.
The Ohio State University
Columbus, Ohio 43210

METHODS AND MATERIALS

Soils Used

Celina silt loam and Brookston silt loam soils were used in all experiments. These soils were chosen because they differed in their organic matter contents. Selected chemical and physical properties of the two soils are given in Table 7.1. Brookston soil was collected from the Ohio State University farm and the Celina soil was collected from the Western Branch, OARDC, South Charleston, Ohio, in the fall of 1979. Both soils were brought to the laboratory and passed through a 2 mm sieve. The soils were stored at field moisture in plastic bags at 4 C until used.

Sewage Sludge Used

A liquid anaerobically digested sewage sludge (Jackson Pike treatment plant, Columbus) was used in all the experiments. Three monthly composited samples were used in the experiments. Characteristics of the sludge for each month are shown in Table 7.2. The sludge contained 3.98 mg/kg of PCB.

Biodegradation of ^{14}C-PCB From Sewage Sludge Amended Celina and Brookston Soils

Preparation of ^{14}C-labeled PCB Solutions--

Two microliters of PCB (Aroclor 1254, obtained from Analabs, Inc., Lot. No. J147A) was added into a screw-capped silanized Teflon-lined 1.8 ml vial (Supelco, Inc.) containing 500 µl hexane to achieve a final concentration of 6 µg PCB/µl. The density of PCB is 1.505 g/cm^3 at 15 C. To three screw-capped silanized Teflon-lined 1.8 ml vials were added 93.3, 18.3, and 3.3 µl of the PCB solution prepared above. A solution of polychlorinated (U-C^{14}) biphenyl (^{14}C-Aroclor 1254, specific

TABLE 7.1. SELECTED PHYSICAL AND CHEMICAL PROPERTIES OF EXPERIMENTAL SOILS

	Particle Size Analysis				Exchangeable Cations				Organic Carbon	0.33 Atm. Water Content
	Sand	Silt	Clay		K	Ca	Mg	P		
	%			pH	kg/ha				(%)	(% by wt.)
Brookston (sil) (Typic Argiaquoll)	15.7	57.7	26.6	7.0	312	6126	1029	75	2.5	25.9
Celina (sil) (Aquic Hapludalf)	12.9	66.4	20.7	7.2	198	3147	968	39	0.8	23.6

TABLE 7.2. CHEMICAL ANALYSIS OF COLUMBUS JACKSON PIKE SEWAGE SLUDGE

Date of Sample	Solids (%)	pH	NH_3-N	Total Kjeldahl-N	Total Phos.	K	Cu	Cd	Pb	Ni	Zn	Cr
			--				mg/kg					--
July, 1980	3.5	8.1	6,032	29,995	21,713	4,673	570	51.71	762	546	5,484	536
September, 1980	5.1	7.5	5,611	29,498	23,862	3,577	595	57.83	1,159	586	7,133	1,491
February, 1980	3.1	8.2	8,762	48,704	24,846	3,768	729	95.50	565	370	5,145	776

activity = 37 µCi/mmol, formula weight = 326, obtained from Amersham Corporation, CFA.586) was prepared by diluting an original batch of 25 µCi PCB in 250 µl of hexane:toluene (9:1 v/v) solution into a silanized liquid scintillation vial (Kimble) with 4.75 ml of hexane to achieve a final concentration of 0.005 µCi ^{14}C/ul. Radiochemical purity of ^{14}C-PCB was determined by the manufacturer. To each of the three vials mentioned above was added 450 µl of the radioactive PCB solution. Hexane was added to each vial to reach a final volume of 550 µl. The vials contained three ^{14}C-labeled solutions of 2.25 µCi/580.1 µg PCB, 2.25 µCi/130.1 µg PCB, and 2.25 µCi/40.1 µg PCB.

All samplings and transfers of PCB were made with Gilson Pipetman digital microliter pipettes (Rainin Instrument Co. Inc.). The vials containing the PCB solutions were covered with aluminum foil to prevent photolysis and kept at -20 C to prevent volatilization of the PCB's. Sylon-CT (Supelco, Inc.) was used to silanize glassware used in the preparation of PCB solutions. The radioactivity of all the ^{14}C-labeled PCB solutions were verified by counting 1 or 2 µl of the radioactive solution in a 15-ml liquid scintillation cocktail.

Preparation of ^{14}C-labeled PCB Amended Sewage Sludge--

Four sub-samples of sludge equivalent to 0.9 g dry sludge (26.1 g wet sludge) (July, 1980 samples) were weighed out into wax paper containers (474 ml cup). The labeled PCB solutions prepared above were added to three of the sub-samples and thoroughly mixed using a plastic spoon. This gave a final concentration of 0, 44.5, 144.5, and 644.5 µg PCB/g dry sludge with a ^{14}C-PCB concentration equivalent to 2.5 µCi ^{14}C/g dry sludge. (A calculation error led to the addition of more nonlabeled PCB than the planned concentration of 25, 125, and 625 µg PCB/g dry sludge).

Amendment of Soils With Sewage Sludge Containing ^{14}C-labeled PCB--

Eighty gram (dry weight) quantities of the two soils (Celina and Brookston) were weighed out on each of eight pieces of wrapping paper of known weight (approx. 16 g each). PCB-sludge mixtures (prepared above) equivalent to 0.4 g dry sludge were transferred to the surface of each soil sample on the paper sheets. This gave a final sludge concentration equivalent to 11.2 metric tons/ha. The sludge was allowed to air dry on the soil and then mixed thoroughly with the soil using a spatula. Subsamples of the soil-sludge mixtures equivalent to 20.1 g (dry weight) were added to each incubation flask and adjusted to field capacity by weighing the container and adding the required amount of distilled water. All treatments were replicated three times. The flasks were sealed and incubated in a Sherer Environmental Control Room at 30 C for 16 weeks. Evolved $^{14}CO_2$ was collected by flushing on a weekly basis as described below.

Incubation and Trapping System--

An incubation and trapping system designed and described by Marinucci and Bertha (1979) was used to prevent expected volatility of PCB from the soil-sludge mixtures or soil-PCB mixtures. A detailed description of the system follows: Samples were incubated in a closed flask with glass, stainless steel, and Teflon components only. The incubation flask shown in Figure 7.1 consisted of a 125-ml micro-fernbach flask (Bellco, Vineland, N.J.) closed with a Teflon-lined screw cap. Holes were drilled in the screw cap and two 16-gauge syringe needles were inserted through the Teflon lining. The syringe needles were secured to the screw cap with epoxy cement and, when not in use for flushing were closed with 0-size polyvinylchloride stoppers (Caplugs Protective Closures Inc., Buffalo, N.Y.). Every week, each incubation flask was evacuated and flushed with air for 5 min. using a water aspirator. Each flushing replaced approximately 99 percent of the original atmosphere, assuming complete mixing (Marinucci and Bertha, 1979). The headspace gas during evacuation and flushing contained radio-labeled PCB vapors and $^{14}CO_2$ which were collected in the trapping unit illustrated in Figure 7.2. The connection between the incubation flask and trapping unit was made with Teflon spaghetti tubing. The volatile PCB and its degradation products other than CO_2 were trapped in units A1 and A2, containing toluene based universal scintillation cocktail prepared in the laboratory as described later. Only the contents of A1 were routinely counted, A2 serving only as a backup trap. $^{14}CO_2$ was then trapped in units 01 and 02 containing phenethylamine (CarboSorb, Packard Inst., Co. Inc.). Both $^{14}CO_2$ trapping units were routinely counted. Units T1 and T2 were kept empty as insurance against loss or mixing of trap contents by back pressure.

The trapping unit was constructed of 24 mm diameter glass scintillation vials. Vial caps and stainless steel tubing connections were all permanently mounted on a common bracket by epoxy cement. Counting vials filled with the appropriate counting fluid were attached or detached by screwing them into the cap threads. This dual trapping system separated volatile ^{14}C-PCB from $^{14}CO_2$. Backflow problems were eliminated by connecting the system starting at the source of vacuum (trap 02) and proceeding upstream to the incubation flask. Disconnection proceed in reverse order, starting in the incubation flask and proceeding through to the vacuum source. This system was successfully used for 16 week incubation periods while studying the fate of ^{14}C-Aroclor 1254 in sewage sludge amended and unamended soils.

Biodegradation of ^{14}C-PCB From Celina and Brookston Soils

This experiment was done to determine the rate of decomposition of Aroclor 1254 in soils without sewage sludge. The same procedures were followed as in the previous experiment except that this time the correct amount of PCB (2.25 µCi/562.5 µg PCB, 2.25 µCi/112.5 µg PCB, and 2.25 µCi/22.5 µg PCB) were added to the three screw capped silanized

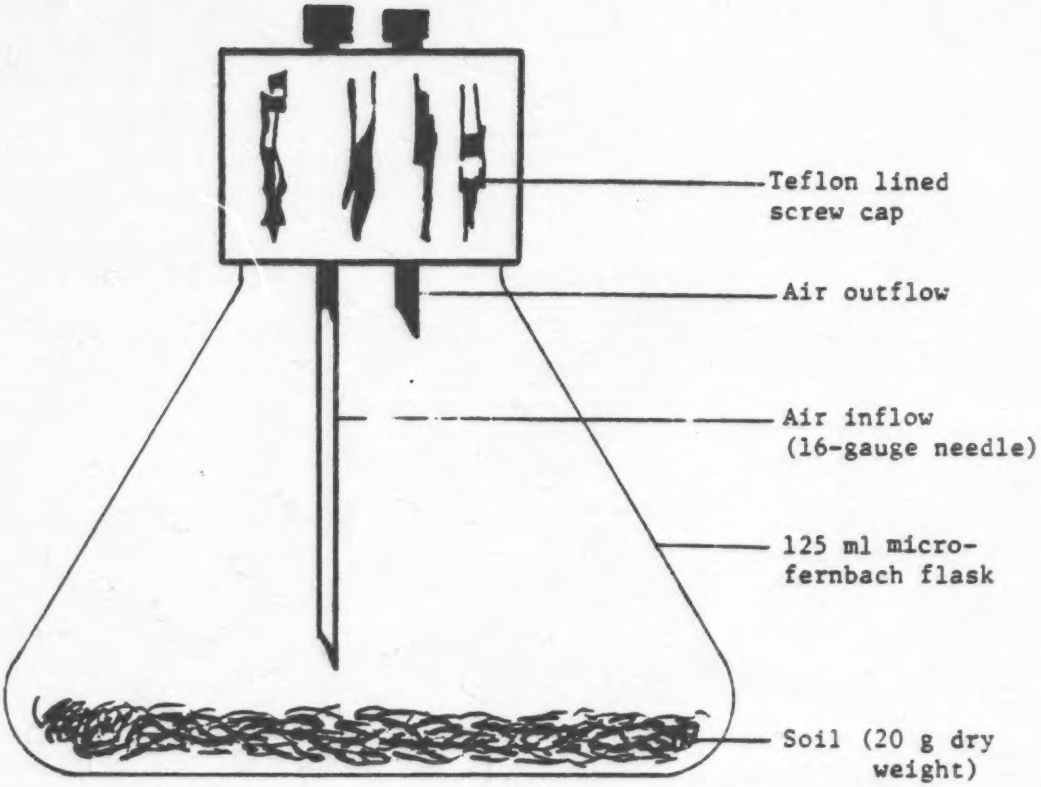
Teflon lined screw cap
Air outflow
Air inflow (16-gauge needle)
125 ml micro-fernbach flask
Soil (20 g dry weight)

Figure 7.1. Soil incubation vessel.

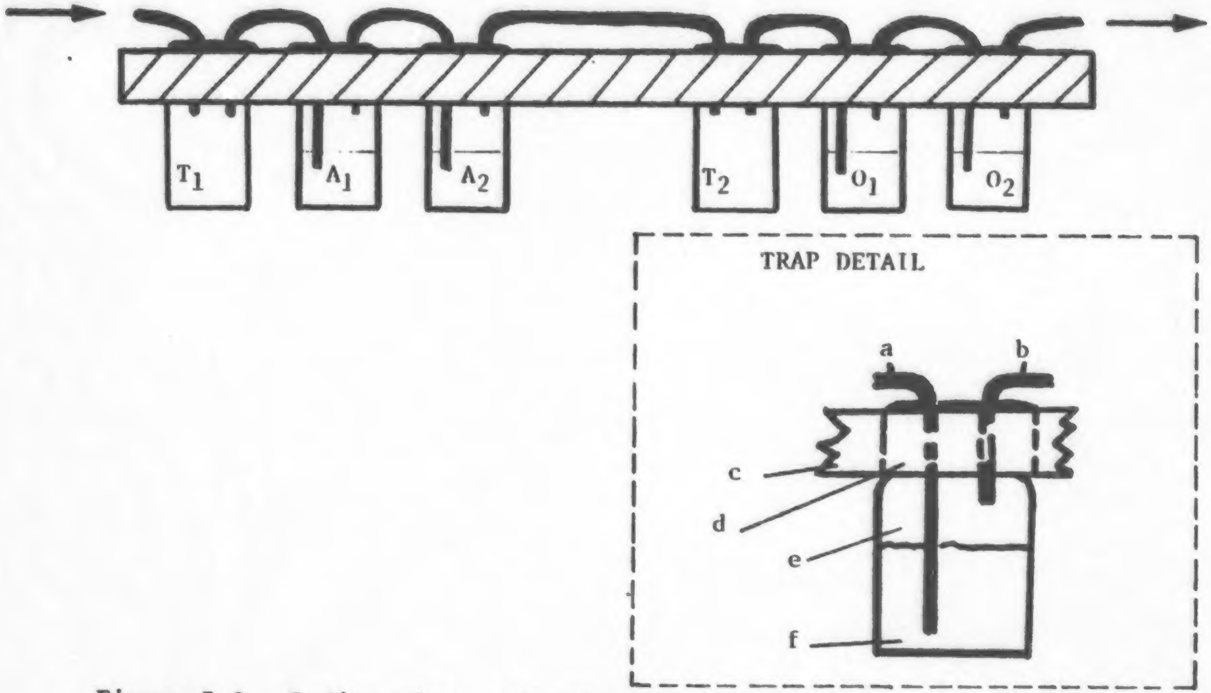

Figure 7.2. Radioactive polychlorinated biphenyl and CO_2 trapping system. T_1 and T_2 are backflow taps. A_1 and A_2 contain a toluene based fluor for polychlorinated biphenyl trapping. O_1 and O_2 contain phenethylamine fluor for CO_2 trapping. Trap detail: (a) inflow (18-gauge stainless steel); (b) outflow (18-gauge stainless steel); (c) 0.625 in. (ca. 1.6 cm) wood mounting board; (d) epoxy-cemented scintillation vial cap; (f) trapping fluor.

1.8 ml vials and diluted to a final volume of 1100 µl of hexane. Half of this solution (550 µl) was added in the center of the soil surface of 80 g (dry weight) each of Celina and Brookston soil contained on wrapping paper of approximately 9 g weight. This small amount of soil was then thoroughly mixed with the remainder of the soil using a spatula. Subsamples of soil equivalent to 20 g (dry weight) were added to each incubation flask and adjusted to field capacity by weighing the container and adding the required amount of distilled water. All treatments were replicated three times. Incubation and flushing were done as in the previous experiment.

Sewage Sludge Decomposition in the Presence of Different Rates of Nonlabeled PCB in Celina and Brookston Soils

Preparation of PCB Solutions--

Six microliters of PCB (1.505 g/cm^3 at 15 C) was added to a screw-capped silanized Teflon-lined 1.8 ml vial containing 500 µl of hexane to achieve a final concentration of 18 µg PCB/µl. To three screw capped Teflon lined 1.8 ml vials was added 233.6, 46.7 and 9.3 µl of the above solution. Hexane was added to each vial to reach a final volume of 550 µl. The vials then contained 168.8 µg PCB/316.4 µl hexane, 843.8 µg PCB/503.3 µl hexane and 4218.8 µg PCB/540.7 µl hexane.

Preparation of PCB Amended Sewage Sludge--

Four sub-samples of sludge equivalent to 6.75 g dry sludge (195.4 g wet sludge) (July, 1980 samples) were weighed out into wax paper containers (474 ml cup). Three subsamples of sludge were thoroughly mixed with the PCB solutions prepared above using a plastic spoon. This gave a final concentration of 0, 25, 125, and 625 µg PCB/g dry sludge.

Amendment of Soils With Sewage Sludge Containing PCB--

Six hundred gram quantities (dry weight) of two soils (Celina and Brookston) were weighed out on each of eight pieces of wrapping paper of approximately 15 g each. The PCB-sludge mixture prepared above equivalent to 3.0 g dry sludge were weighed out on the soil surface contained on each paper. The sludge was allowed to air dry on the soil and then mixed thoroughly with the soil using a spatula. The final concentration of sludge was equivalent to 11.2 metric ton/ha. Sub-samples of the amended soils equivalent to 150.75 g (dry weight) were added to screw-capped wide mouth (10.5 cm high x 8.0 cm diameter) jars and adjusted to field capacity by weighing the container and adding the required amount of distilled water when needed. A 25 ml beaker containing 25 ml of 0.5 N sodium hydroxide was placed on the soil surface in the center of each jar. Three replications per rate were used. The soils were incubated at room temperature for 30 days. Evolved CO$_2$ was determined at regular intervals by titration of the unreacted NaOH. For titration, the NaOH solution was transferred to a 100 ml beaker, the carbonate precipitated as BaCO$_3$ with 5.0 ml of a saturated solution of barium chloride, and finally titrated with 0.5 N hydrochloric acid using phenolphthalien as an indicator. The results were expressed as mg CO_2-C produced per 100 g dry soil.

Uptake of ^{14}C-PCB by Kentucky 31 Fescue From Sewage Sludge Amended Celina and Brookston Soil

Preparation of ^{14}C-labeled PCB Solutions--

A ^{14}C-PCB solution was prepared by diluting an original batch of 25 µCi/250 µl hexane:toluene (9:1 v/v) solution with 7.75 ml hexane in a liquid scintillation vial containing a previously diluted radioactive solution of 1.85 ml of 0.005 µCi ^{14}C/µl. This mixture was calculated to give a final concentration of 0.004 Ci ^{14}C/ 1. (Verification of the radioactivity of this PCB solution gave a concentration of 0.007 µCi ^{14}C/µl. This concentration was used for all calculations). To each of three silanized liquid scintillation vials were added 3.3 ml of the radioactive solution prepared above and 1.3, 6.8, and 34.2 µl of nonlabeled PCB to achieve a final concentration of 23.0 µCi/2165 µg PCB, 23.0 µCi/10415 µg PCB, and 23 µCi/51665 µg PCB.

Preparation of ^{14}C-labeled PCB Amended Sewage Sludge--

Four sub-samples of sludge equivalent to 82.5 µg of dry sludge (1617.7 g wet sludge) (September, 1980 samples) were weighed out in polyethylene cone-shaped plastic containers (15.0 cm high x 12.5 cm diameter). Three subsamples of sludge were thoroughly mixed with the labeled PCB solutions prepared using a plastic spoon. This gave a final concentration of 26.3, 126.3, and 626.3 µg PCB/g; dry sludge with a ^{14}C-PCB concentration equivalent to 0.278 µCi ^{14}C/g dry sludge.

Amendment of Soils With Sewage Sludge Containing ^{14}C-labeled PCB--

Fifteen hundred gram quantities (dry weight) of two soils (Celina and Brookston) were weighed out in cone-shaped polyethylene plastic containers (16.5 cm high x 14.5 cm diameter) lined with polyethylene plastic bags. The ^{14}C-PCB-sludge mixture prepared above, equivalent to 7.5 g of dry sludge, were weighed out on the soil surface within each container. This gave a final sludge concentration of 11.2 metric ton/ha. Five replicates per treatment were used for each soil. The sewage sludge was allowed to infiltrate into the soil and air dry. The soil surface was then manipulated by mixing the top 4 cm of the soil surface with a spatula to simulate tillage incorporation of the sludge into the soil. All pots were sown with 0.65 g seeds/pot of fescue (*Festuca arundinacea* Schrib. "Kentucky 31"). The pots were placed in a growth chamber (16 hr day, 37 C day, 20 C night, 70-80 percent humidity, and 3900-7990 foot candles at a height of 80 cm). All pots were watered on alternate days with distilled water until the soil was saturated at the surface.

Determination of Plant Uptake of PCB--

Kentucky 31 fescue was allowed to grow until about 10-15 cm in height. At this time the fescue was cut to about a 2.5-cm height using a single edged razor blade. The harvested material was transferred to paper bags and dried at 70 C overnight and weighed. The dried plant material was ground using a Wiley Mill, Intermediate Model, 3383-L40.

Sub-samples of the ground plant material (two/pot) were weighed (0.05-0.25 g) in a Combusto-Cone (Packard Instr. Co. Inc.) containing small amounts of cellulose powder. Samples were oxidized with a Packard Sample Oxidizer[1], Model 306. The resulting gases were collected and adsorbed in a scintillation cocktail (Oxiprep-2 and Oxisorb-CO_2 (11:7), obtained from New England Nuclear). Uptake of ^{14}C-PCB by fescue was followed on approximately monthly intervals following the procedure outlined above.

Leaf Absorption Studies of ^{14}C-PCB

Preparation of ^{14}C-labeled PCB Solution--

To a screw-capped silanized Teflon-lined 1.8 ml vial were added 8.8 µl of nonlabeled PCB and 135 µl of ^{14}C-PCB from a previously diluted radioactive solution of 0.005 µCi ^{14}C/µl. Hexane was added to reach a final volume of 1100 µl. The vial then contained 0.675 µCi/13252 µg PCB.

Preparation of ^{14}C-labeled PCB Amended Sewage Sludge--

Two subsamples of sludge equivalent to 21.2 g dry sludge (684 g wet sludge) (February, 1981 samples) were weighed out in polyethylene cone-shaped plastic containers (16.5 cm high x 14.5 cm diameter). One subsample was thoroughly mixed with the labeled PCB solution prepared above using a plastic spoon. This gave a final concentration of 0 and 625 µg PCB/g dry sludge with a ^{14}C-PCB concentration equivalent to 0.03 µCi ^{14}C/g dry sludge.

Amendment of Leaf With Sewage Sludge Containing ^{14}C-labeled PCB--

Application of sewage sludge by spray irrigation to plant leaf surfaces was simulated by "painting" liquid sewage sludge prepared as described above on fescue leaf surfaces with a nylon brush. This amended liquid sewage sludge was added in small amounts in several applications allowing it to dry between applications to avoid excess dropping of the sludge from the leaf surface. The sludge application rate was equivalent to 2.25 metric ton/ha. The fescue used was that growing in sludge amended Brookston silt loam and had been used previously for the plant uptake studies of ^{14}C-PCB. Both ^{14}C-PCB labeled sludge and non-labeled sludge were used and each treatment was replicated five times.

Three days after sludge application to leaf surfaces, the plant materials was harvested by cutting with a single edged razor blade. Half of the plant samples were washed with an aqueous solution (0.1 percent) of dodecyl sodium sulfate detergent to remove adhering sludge. The harvested material was ground with an agate mortar and pestle. This was done to prevent volatilization of PCB's from the sludge during oven drying.

Sub samples of the ground plant material (two/pot) were weighed (0.15-0.37 g) in a Combusto-Cone containing small amounts of cellulose powder. Samples were oxidized as described in the previous plant uptake experiment.

Scintillation Counting

Carbon activity was determined using a Beckman Liquid Scintillation Counter, Model 100 C. Counting times were 20 minutes, at a 2.0 percent standard error, using the internal standard method for quench correction. The internal standard used was a toluene ^{14}C standard ($5.27 \times 10^5 \pm 3.2$ percent DPM/g) obtained from Packard Inst. Co. Inc. (Cat. No. 6004062). Two hundred microliters of this standard, equivalent to 9.4×10^4 (disintegrations per minute) DPM were added to randomly selected vials to check counting efficiency of the instrument and to calculate quenching using an internal standard procedure.

In the biodegradation studies, solutions of ^{14}C-Aroclor 1254 were counted in a 15 ml cocktail prepared in the laboratory by adding toluene to 0.150 g/l POPOP (1,4-bis-2-(5-phenyloxazolyl)- Benzene, Scintillation Grade) and 4 g/l POP (2,5-Diphenylozazole, Scintillation Grade) obtained from Packard Inst. Co. Inc. $^{14}CO_2$ was counted in a 2:1 solution of the liquid scintillation fluor prepared above (10 ml) and 5 ml of phenethylamine based Carbo-Sorb carbon dioxide absorber (Packard Inst. Co. Inc.). Counting efficiency ranged from 88 to 97 percent for the 15 ml cocktail toluene-based scintillation fluor prepared as described above and 69-86 percent for the 2:1 solution. In the plant uptake and leaf absorption studies the $^{14}CO_2$ was trapped in Oxiprep-2 liquid scintillation fluor (11 ml) and Oxisorb-CO_2 (7 ml), obtained from New England Nuclear.

Analysis of the Data

One way analysis of variance (ANOVA) was performed on the data from all the experiments. If a significant difference was found, the Least Significant Difference (LSD) procedure was used to show differences in the treatment means. Additionally, in the plant uptake experiment the two way analysis of variance was carried out to evaluate any difference between the two soil types used (Celina and Brookston) related to the concentration of PCB used. Results of the statistical analyses are shown in the Appendix Tables. The statistical analysis was done on a Texas Instrument electronic calculator TI programmable 58/59.

RESULTS AND DISCUSSION

Biodegradation of ^{14}C-PCB in Celina and Brookston Soils When Incorporated With and Without Sewage Sludge

The quantity of $^{14}CO_2$ evolved when ^{14}C-PCB was added to the Celina soil with and without sewage sludge were very similar (Figures 7.4 and 7.6). However, with Brookston soil, $^{14}CO_2$ evolution from ^{14}C-PCB was around 10 times higher when applied without sewage sludge (Figures 7.3 and 7.5). No effective explanation of these anomolous data is readily apparent. However, it is possible that it is related to the organic matter content of the Brookston soil and to the protection of PCB by the sewage sludge.

An inverse relationship was found between the concentration of ^{14}C-PCB added to both the Brookston and Celina soils and the quantity of evolved $^{14}CO_2$. This relationship was true regardless of whether the ^{14}C-PCB are added with the sewage sludge or without the sewage sludge (Figures 7.3-7.6). This relationship was particularly evident for the lowest rate of ^{14}C-PCB and was not as evident at the two highest concentrations. These results suggest a stimulation-inhibition effect of the PCB's on the soil microbial population.

To test the possibility of a concentration effect of PCB on stimulation or inhibition of the soil microflora, an additional study was done in which the degradation of sewage sludge carbon in the presence of different rates of nonlabeled PCB were examined in both experimental soils. The data showed no effect of PCB concentration on the degradation rate of sewage sludge on both Celina and Brookston soils (Figures 7.7 and 7.8). It seems, therefore, that the concentration of applied PCB's only affected specific soil microorganisms capable of degrading PCB's but not components of the general soil microbial population. No attempt was made to differentiate this PCB-sensitive microflora on both experimental soils. Pal et al. (1979) reported that the growth of certain microorganisms may be stimulated by low levels of PCB's (i.e., 0.1 µg/g of soil weight) without apparent influence on soil respiration while at concentrations greater than 1 percent of soil weight, the soil biological activity is inhibited. The nature of this experiment does not allow us to predict with assurance whether stimulation by low concentration of PCB or inhibition by high concentrations was responsible for the results obtained. However, it seems that an inhibition of specific soil microorganisms capable of degrading PCB by the higher concentration of PCB was more probable than a stimulation effect.

Volatilization of ^{14}C-PCB in Celina and Brookston Soil--

It has been found by several investigators (Haque et al., 1974; Iwata et al., 1973; Gresshoff et al., 1977; Hiralzum et al., 1979) that the affinity of PCB's for adsorption increases with the organic matter

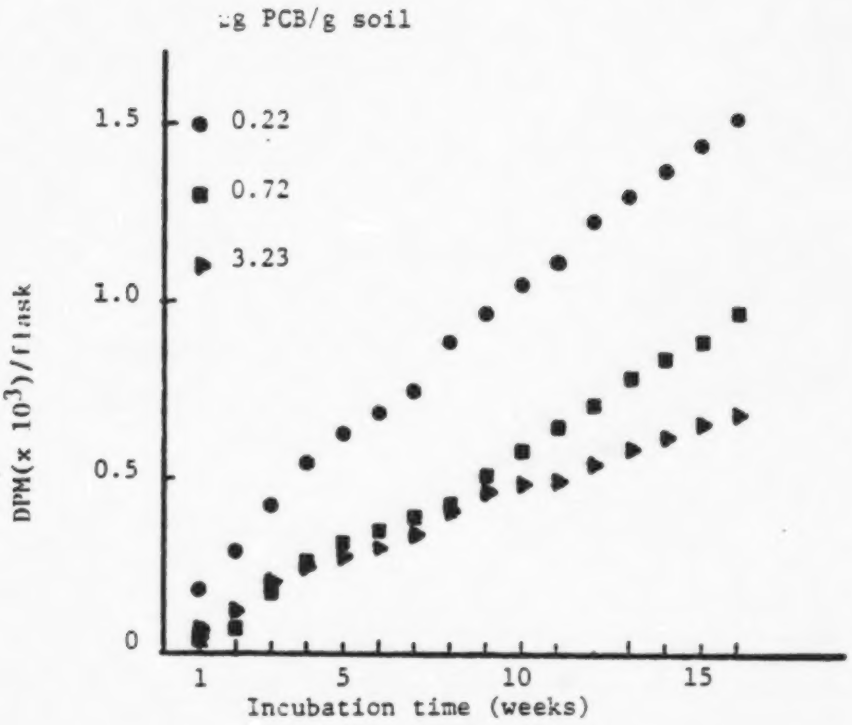

Figure 7.3. $^{14}CO_2$ evolution from ^{14}C-PCB sewage sludge amended Brookston soil.

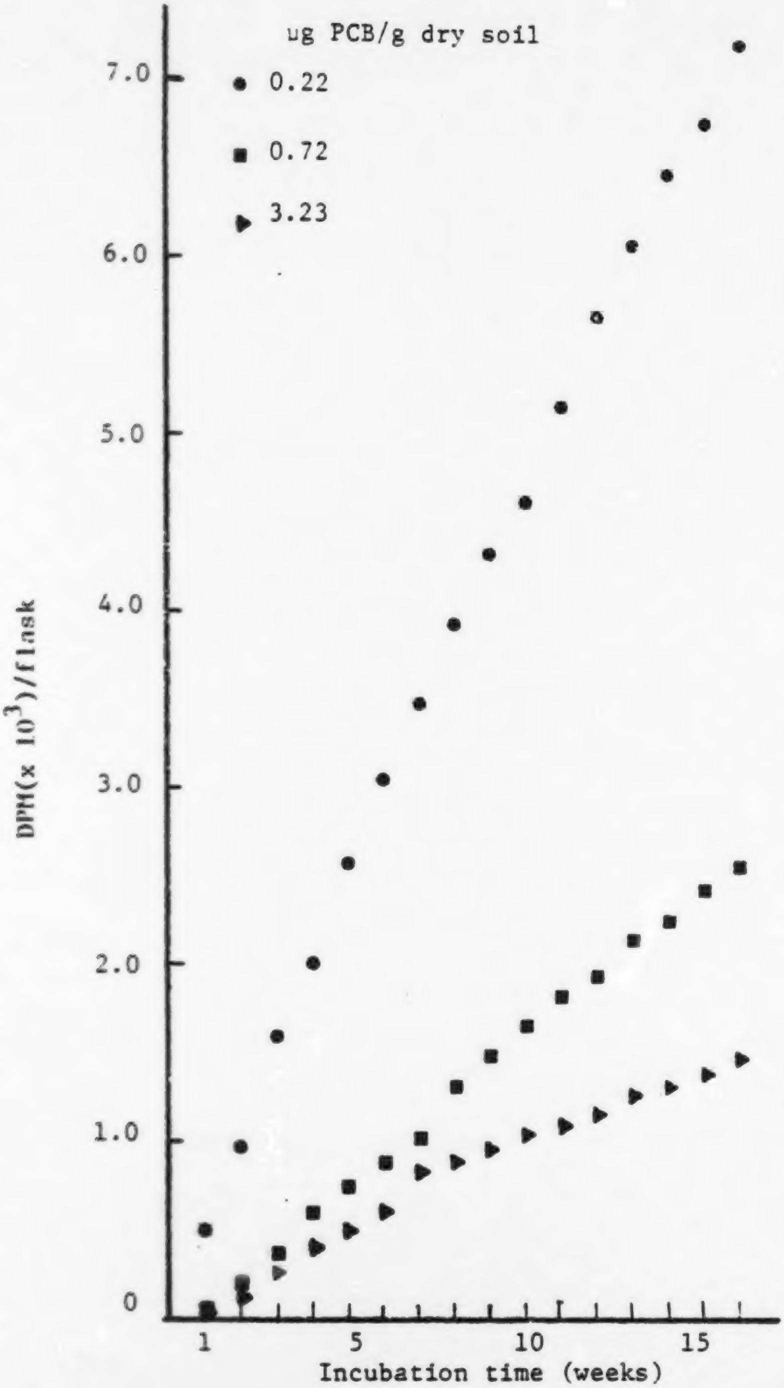

Figure 7.4. $^{14}CO_2$ evolution from ^{14}C-PCB sewage sludge amended Celina soil.

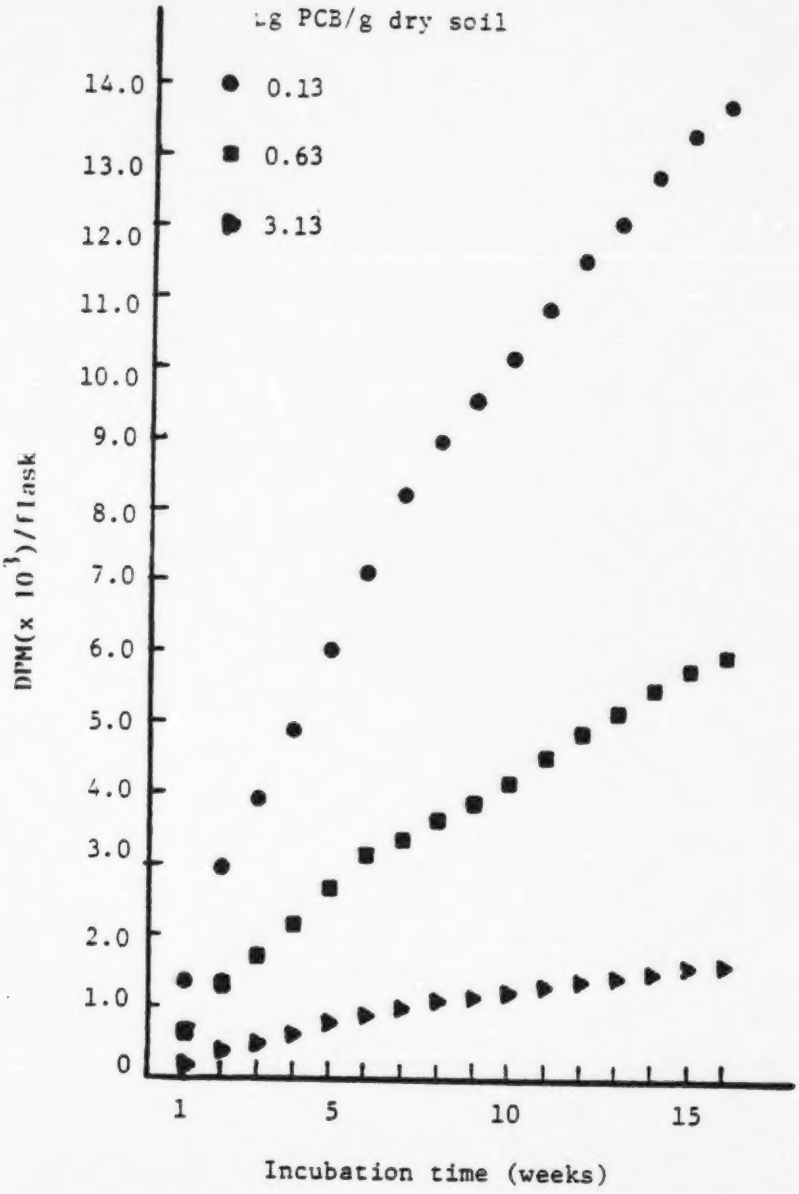

Figure 7.5. $^{14}CO_2$ evolution from ^{14}C-PCB amended Brookston soil.

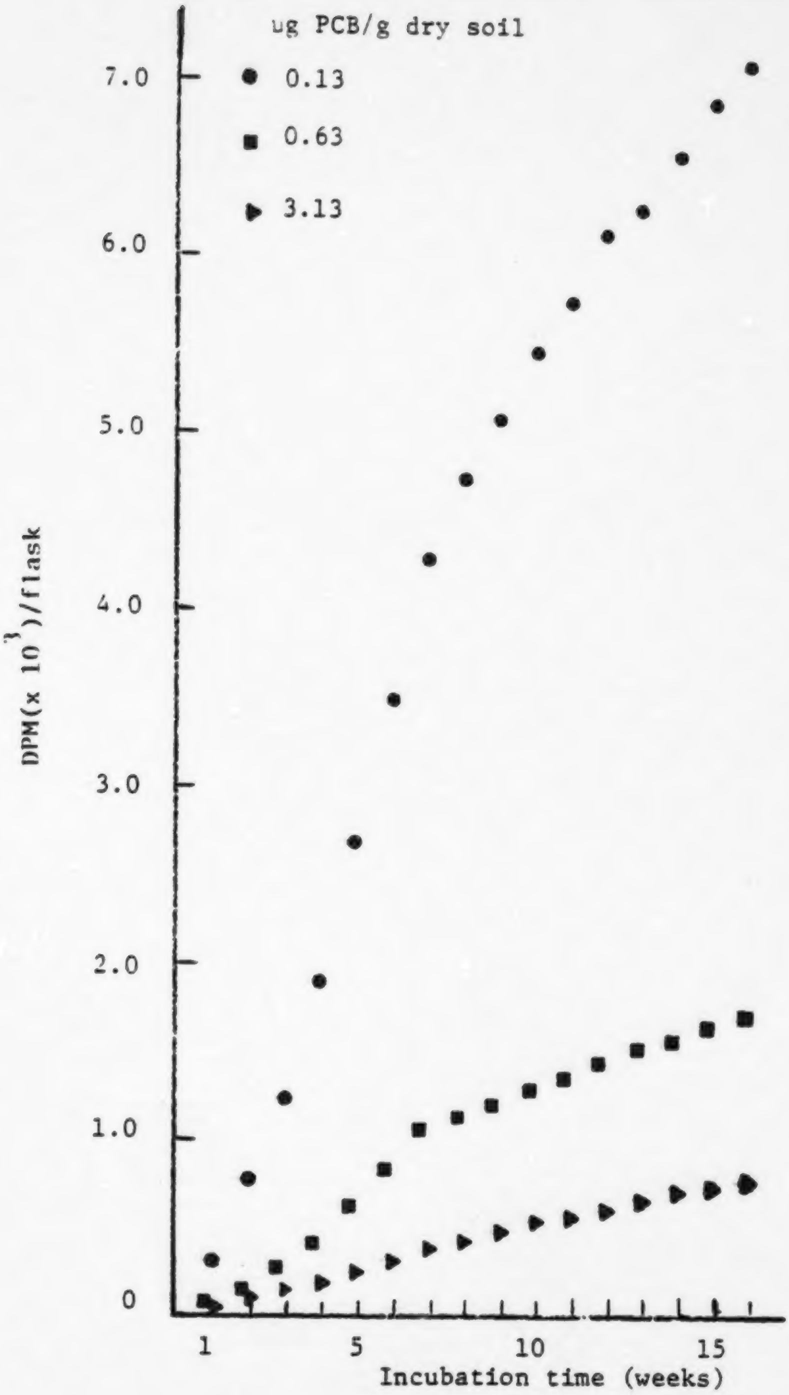

Figure 7.6. $^{14}CO_2$ evolution from ^{14}C-PCB amended Celina soil.

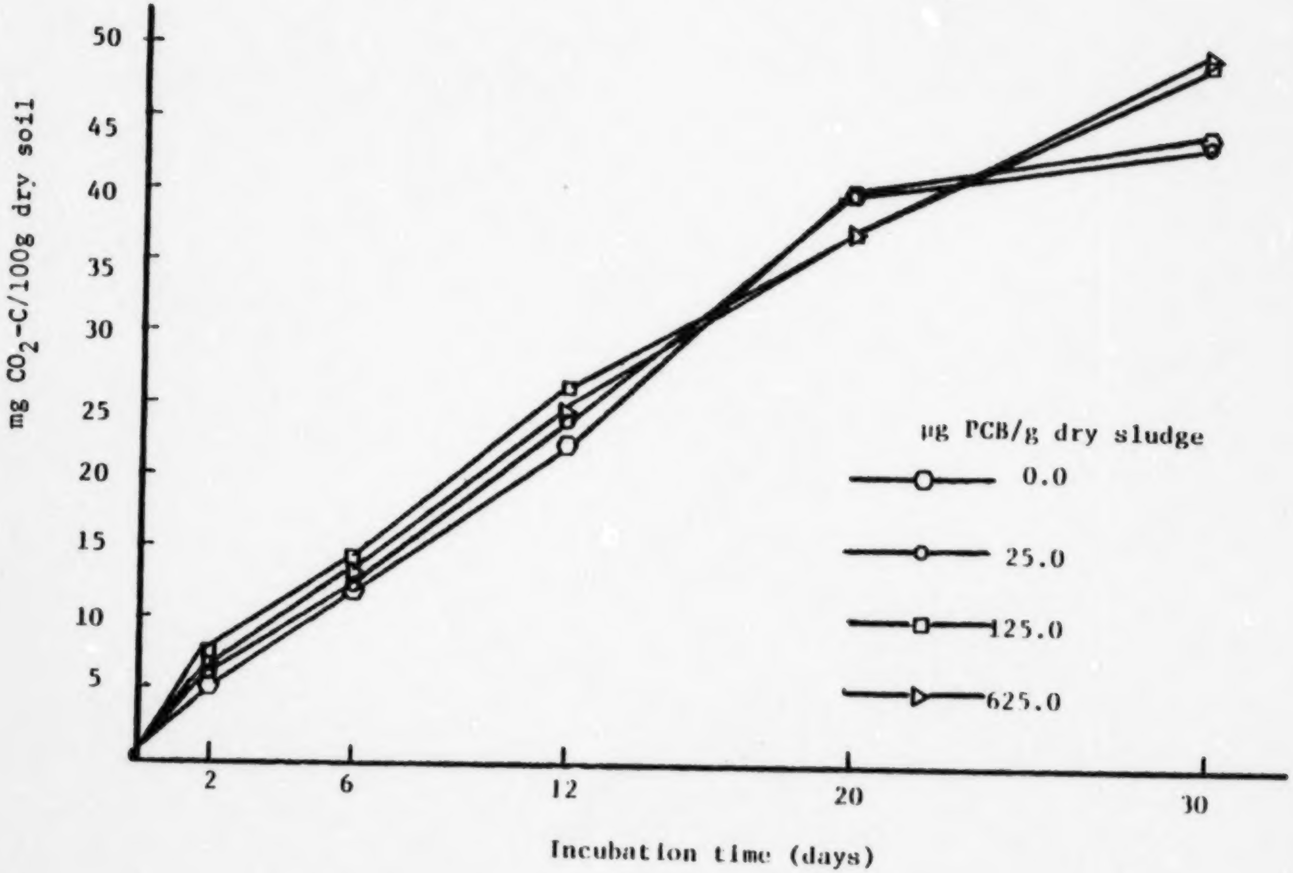

Figure 7.7. Cumulative CO_2-C evolved from PCB sewage sludge amended Brookston soil.

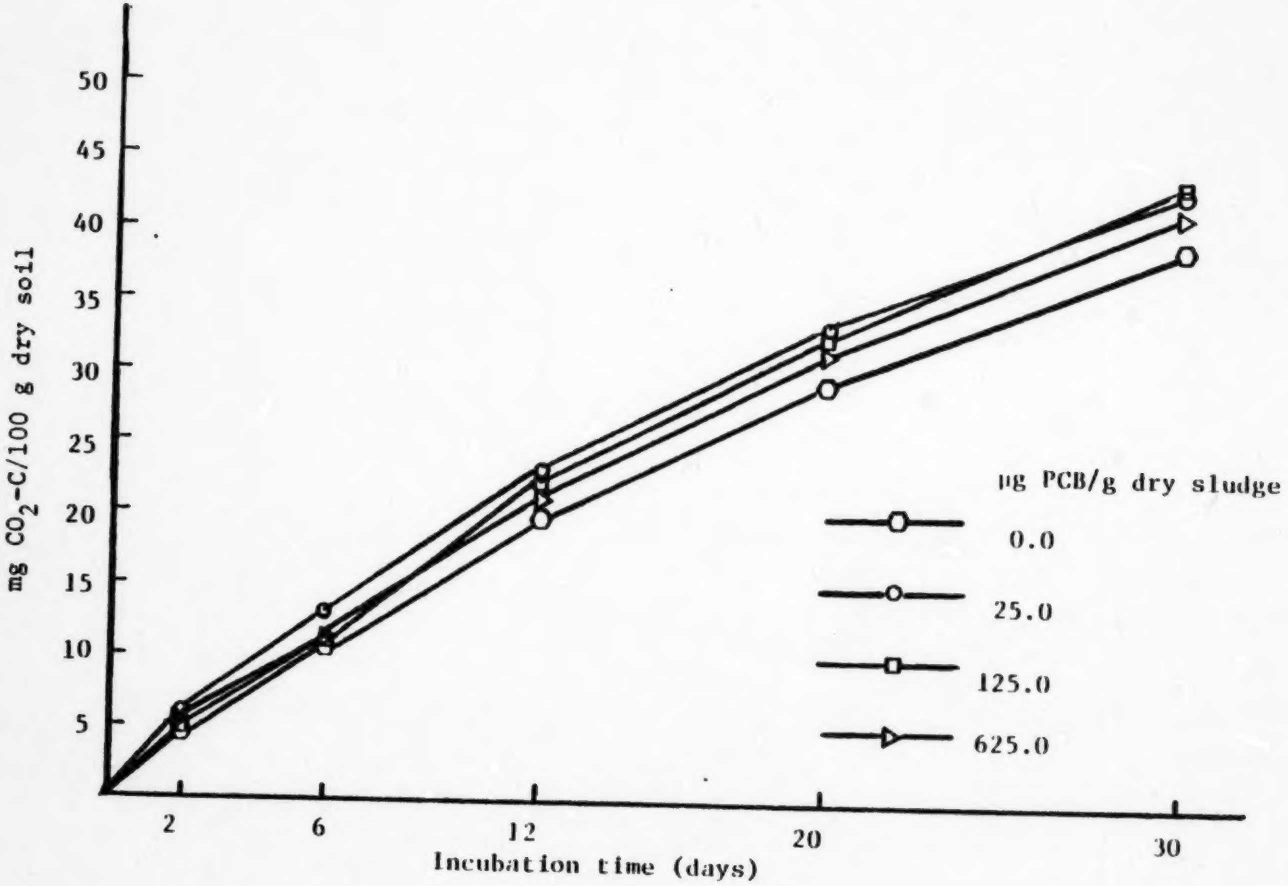

Figure 7.8. Cumulative CO_2-C evolved from PCB sewage sludge amended Celina soil.

and clay contents of the soil and also as the number of chlorine atoms on the isomer increases. It can be assumed that these factors may decrease PCB volatilization by reducing the activity of PCB's in the soil (Pal et al., 1980). The fact that Brookston soil contains higher amount of organic matter than the Celina soil (Table 7.1) may explain why volatilization of Aroclor 1254 is lower for the Brookston soil than from the Celina soil when incorporated both with and without sewage sludge (Figures 7.9-7.12). The effect of sewage sludge in reducing PCB volatilization by adsorption of the PCB's is clearly shown on the Celina soil (Figure 10 and 12) in which more volatilization occurred on the treatment without sewage sludge than the one with sewage sludge. The Brookston soil showed slightly slower volatilization of PCB from the treatment without sewage sludge. This could be because the concentration of PCB applied was less than on the treatment with sewage sludge and to the presence of organic compounds in the sludge that adsorb the PCB's.

Studies on the degradation rate of PCB's by microorganisms have conclusively established that the less chlorinated biphenyls are metabolized more readily and completely than the highly chlorinated species. Similarly, studies on the volatilization of PCB's have shown that the low-molecular weight PCB's tend to volatilize much more readily than the high-molecular weight species. Since Aroclor 1254 possesses a higher percentage of highly chlorinated species, the low pecentage of PCB decomposed or volatilized on both Celina and Brookston soil (Table 7.3) were probably the less chlorinated biphenyls present in the formulation of Aroclor 1254. Pal et al. (1980) state that the lack of dehalogenation capacity of most microorganisms is responsible for the lower degradation rate observed as the level of chlorination of biphenyl increases. The fact that approximately 99.0 percent of the applied PCB's remained in both experimental soils at the end of the incubation period clearly shows the extreme persistence of Aroclor 1254 in soils. This is in agreement with a study done by Iwata et al. (1973) in which they found Aroclor 1254 to be extremely persistent in six California soils over a period of one year with the lesser halogenated biphenyls disappearing faster than the higher ones. This suggest a long-term buildup of higher chlorinated mixtures of PCB's in soils.

The decomposition studies of PCB showed a large variation between replicate flasks. This problem was not as evident in the volatilization data. This problem could be due to improper mixing of the PCB-sludge-soil system. PCB's are extremely lipophilic which would make uniform mixing of soil and sludges which are in an aqueous environment very difficult. An alternative explanation could be that the select group of microorganisms responsible for the PCB degradation were not present at the same level in the small soil samples used.

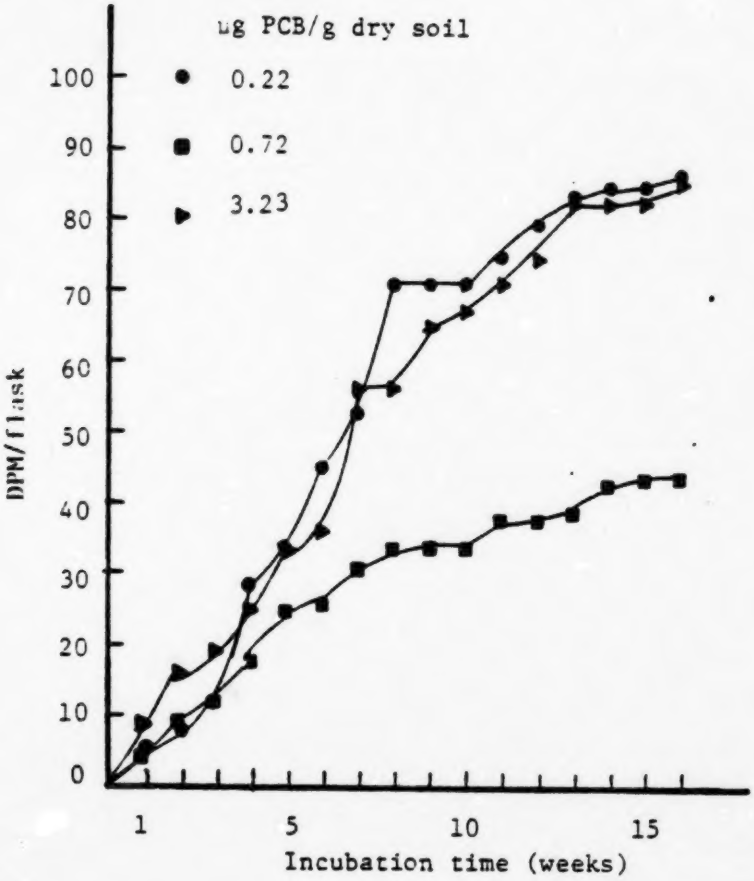

Figure 7.9. Volatilization of ^{14}C-PCB and its degradation products other than $^{14}CO_2$ from ^{14}C-PCB sewage sludge amended Brookston soil.

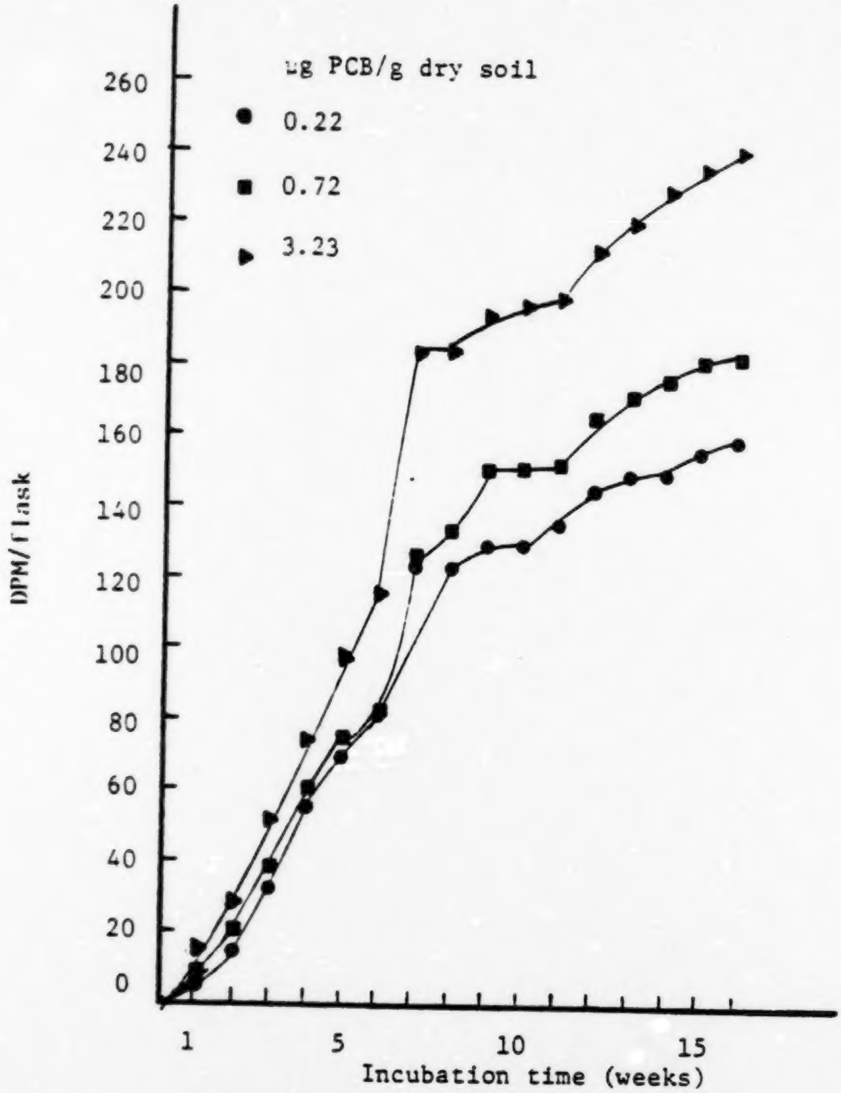

Figure 7.10. Volatilization of ^{14}C-PCB and its degradation products other than $^{14}CO_2$ from ^{14}C-PCB sewage sludge amended Celina soil.

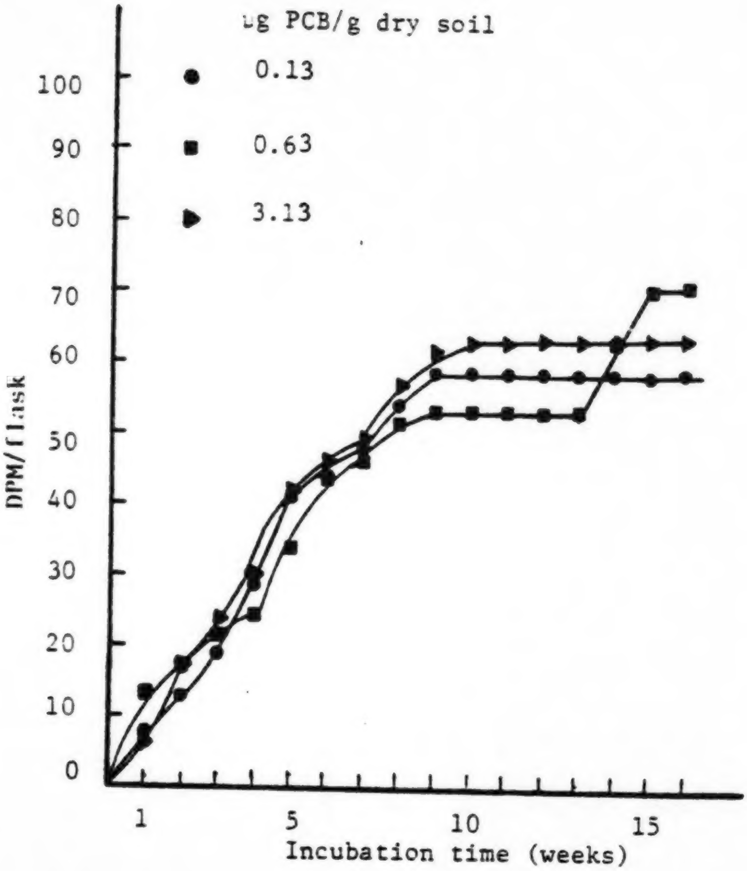

Figure 7.11. Volatilization of ^{14}C-PCB and its degradation products other than $^{14}CO_2$ from ^{14}C-PCB and its degradation products other than $^{14}CO_2$ from ^{14}C-PCB amended Brookston soil.

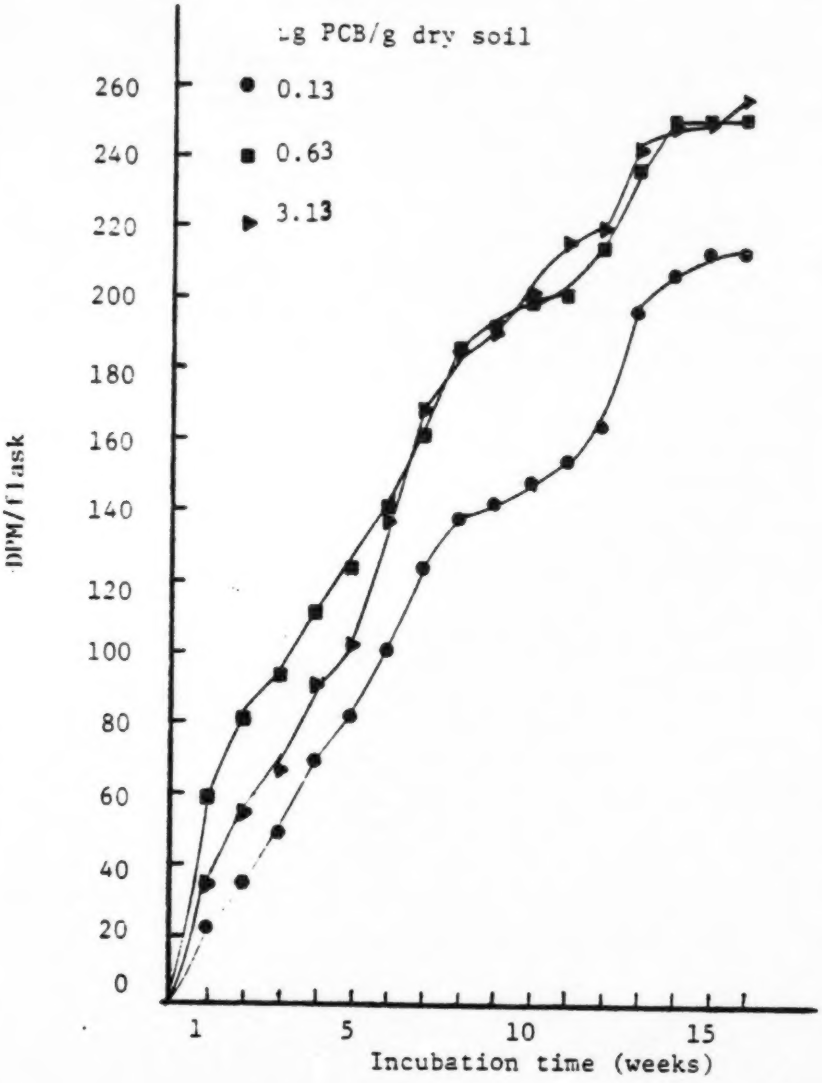

Figure 7.12. Volatilization of ^{14}C-PCB and its degradation products other than $^{14}CO_2$ from ^{14}C-PCB amended Celina soil.

TABLE 7.3. BIODEGRADATION OF ^{14}C-PCB IN CELINA AND BROOKSTON SOILS WHEN ADDED WITH AND WITHOUT SEWAGE SLUDGE DURING 16 WEEKS PERIOD

Treat-ments	Total PCB Added (μg/flask)	$^{14}CO_2$ Evolved (DPM/flask)	$^{14}CO_2$ Evolved (%)*	PCB Evolved as $^{14}CO_2$ (μg/flask)	^{14}C-PCB Volatilized (DPM)	PCB Volatilized (%)*	PCB Volatilized (μg/flask)	Total PCB Remaining in Soil (%)
				Brookston				
With	4.45	1518.5	0.3	0.01	86.6	0.02	<0.01	99.7
Sewage	14.45	973.6	0.2	0.03	43.6	0.01	<0.01	99.8
Sludge	64.45	677.3	0.1	0.08	84.4	0.02	0.01	99.9
Without	2.5	13742.3	2.5	0.06	58.8	0.01	<0.01	97.5
Sewage	12.5	5996.9	1.1	0.14	70.8	0.01	<0.01	98.9
Sludge	62.5	1590.5	0.3	0.18	62.6	0.01	0.01	99.7
				Celina				
With	4.45	4954.1	0.9	0.04	160.8	0.03	<0.01	99.1
Sewage	14.45	1698.1	0.3	0.04	183.9	0.03	0.01	99.7
Sludge	64.45	1465.0	0.3	0.17	240.7	0.04	0.03	99.7
Without	2.5	4944.4	0.9	0.04	160.8	0.03	<0.01	99.1
Sewage	12.5	1395.8	0.3	0.03	251.3	0.05	0.01	99.7
Sludge	62.5	738.5	0.1	0.08	254.8	0.05	0.03	99.8

* Total ^{14}C-PCB added/flask = 5.55×10^5 DPM

Uptake of ^{14}C-PCB by Kentucky 31 Fescue From ^{14}C-PCB Sewage Sludge Amended Celina and Brookston Soil

Fescue plants grown on Celina soils showed slightly higher uptake of PCB at the higher concentration of PCB applied than the fescue plants grown on the Brookston soil (Figure 7.13 and 7.14), however, no significant differences between the two soil types were shown by two way analysis of variance test. The trend in the results are not surprising since Brookston soil contains higher organic matter than the Celina soil which by adsorbing the PCB's make them less available. Also, degradation of ^{14}C-PCB was higher on the Celina soil than on the Brookston soil (Figures 7.3 and 7.4) which may contribute more $^{14}CO_2$ which was fixed by the fescue leaves through photosynthesis. This last observation is supported by the fact that the control fescue plants show some ^{14}C activity which probably comes from fixation of $^{14}CO_2$ as a result of the degradation of ^{14}C-PCB from the Celina and Brookston soils. Data obtained from the biodegradation of PCB on both experimental soils seems to support this last observation since amount of $^{14}CO_2$ evolved is high enough to compensate for this ^{14}C-activity showed on the fescue harvest plant.

The overall uptake of PCB by fescue plants was approximately 0.01 percent (Table 7.4) from all the concentration of PCB applied. This amount of PCB taken up by plants on both experimental soils is still insignificant even when assuming that, in the worst case, all the activity of ^{14}C-measured on the harvest plant came exclusively from ^{14}C-PCB uptake and not from $^{14}CO_2$ evolution from soils and subsequent fixation. Remember, however, that the concentration of PCB added to the sewage sludge in this experiment was higher than the concentration of PCB usually found in municipal sewage system where it ranges from 0 to 23 μg/g (Furr et al., 1976). However, this small amount of uptake of PCB by plants could still be potentially serious due to biomagnification of PCB's on the food chain. This small uptake of PCB in this study is similar to that shown by Weber et al. (1979). In this study Kentucky 31 fescue absorbed up to 0.17 percent of applied Aroclor 1254 in 50 days from Lakeland sand.

Leaf Absorption of ^{14}C-PCB by Kentucky 31 Fescue From Foliar Application of ^{14}C-PCB Sewage Sludge

Table 7.5 shows the results obtained from foliar application of ^{14}C-Aroclor 1254 labeled sewage sludge to Kentucky 31 fescue plants. About 41.5 percent of the applied PCB was present on the fescue leaf surface with the sewage sludge 3 days after applying the sludge. Only 6.2 percent of the applied PCB's was accounted for on the fescue leaves washed with a detergent to remove the sewage sludge particles. It is presumed that this remaining PCB was absorbed on the cuticle and other lipophilic parts on the fescue leaf surfaces. Assuming 100 percent efficiency of the detergent in removing only the sewage sludge particles

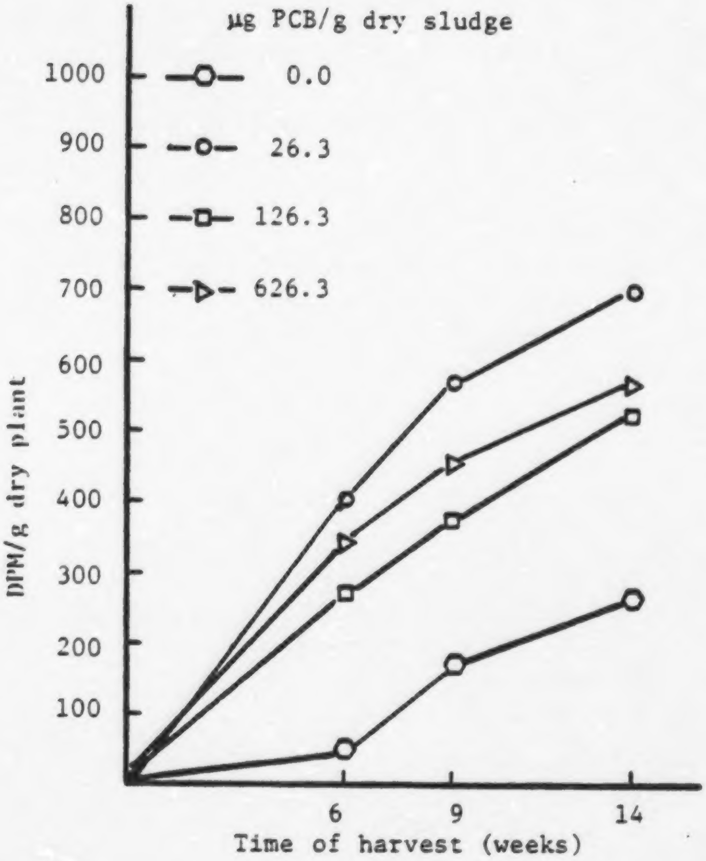

Figure 7.13. Uptake of ^{14}C-PCB by Kentucky 31 fescue from ^{14}C-PCB sewage sludge amended Brookston soil.

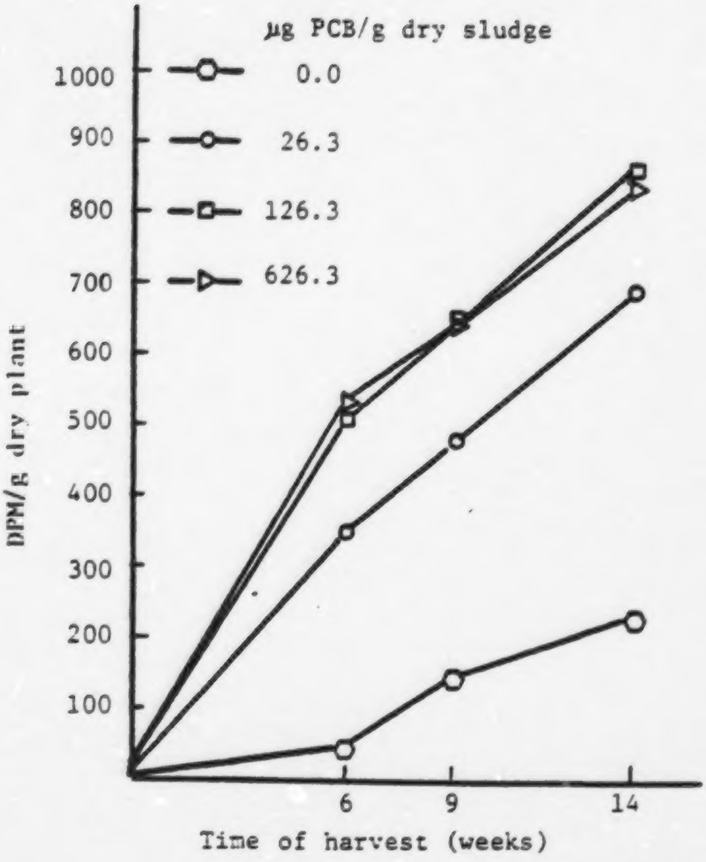

Figure 7.14. Uptake of ^{14}C-PCB by Kentucky 31 fescue from ^{14}C-PCB sewage sludge amended Celina soil.

TABLE 7.4. CUMULATIVE UPTAKE OF ^{14}C-PCB BY KENTUCKY 31 FESCUE FROM ^{14}C-PCB SEWAGE SLUDGE AMENDED CELINA AND BROOKSTON SOIL

Soil	Treatments (μg PCB/g dry sludge)	Total PCB Added (μg/pot)	Cumulative Mean Wt. of Harvested Plant Material (end of 14 weeks) (g/pot)	Cumulative Mean PCB Taken up by Plants (end of 14 weeks) (μg/pot)	Total PCB Remaining in Soil (end of 14 weeks) (%)
Brookston	26.3	197.25	3.96	0.03	99.99
	126.3	947.25	3.95	0.09	99.99
	626.3	4697.25	4.14	0.51	99.99
Celina	26.3	197.25	2.21	0.02	99.99
	126.3	947.25	1.90	0.09	99.99
	626.3	4697.25	2.43	0.80	99.98

TABLE 7.5. LEAF ABSORPTION OF ^{14}C-PCB BY KENTUCKY 31 FESCUE FROM FOLIAR APPLICATION OF ^{14}C-PCB SEWAGE SLUDGE

Treatments (µg PCB/g dry sludge)	Mean Wt. of Harvested Plant Material (g/pot)	Mean DPM of Harvested Plant Material (DPM)	Net DPM of Harvested Plant Material (DPM)	Total PCB Added (µg/pot)	^{14}C-PCB Absorbed (%)*	PCB Absorbed (µg/pot)	Total PCB Unaccounted for (%)
			Washed				
0.0	8.7	371.5					
625.0	8.0	15890.8	15609.3	2208.8	6.2	137.9	93.8
			Not Washed				
0.0	8.7	743.0					
625.0	8.0	104584.0	103841.0	2208.8	41.5	917.5	58.5

* Total activity of ^{14}C-PCB/pot = 2.5×10^5 DPM

from the fescue leaf surface, 35.3 percent of the applied PCB remained in the sewage sludge particles on the fescue leaf surfaces rather than being absorbed by the leaves.

The PCB unaccounted for (58.5 percent of applied) was presumably contained in the sludge that dripped off the leaves on to the soil at the time of application. Alternatively, some could have been lost by volatilization from the fescue leaf surface. In previous work (Moza et al., 1976 and Weber et al., 1979) on foliar application of radioactive PCB directly to the experimental plant, it was found that about 90.0 percent of the applied radioactivity was unaccounted for and was attributed to volatilization. This suggests that adsorption of PCB by sewage sludge particles may actually prevent PCB volatilization from the leaf surface.

Although the levels of ^{14}C-PCB actually absorbed on the fescue leaf surface were still low in comparison to that applied, the results do show that PCB containing sewage sludge did contaminate the fecue plants when applied directly to the growing plant.

The results of these experiments support the current recommendation of the Federal Drug Administration (as reported by Jelineck et al., 1978) that sludge should not be applied directly to growing or mature crops where sludge particles may remain in or on the food. This is especially true when considering management of pasture land because of the potential for contaminated milk and meat that might result from animals eating such feed. It seems then, that a better method of sludge application to pasture or forage plants is after grazing and cutting and before regrowth since it provides an additional safeguard by preventing direct ingestion of sludge particles (Miller et al., 1979).

Although conservative, the current requirement of the Environmental Protection Agency to incorporate into the soil any sludge containing 10 mg/kg PCB or more when applied to land used for producing animal and human food, provides a good control for preventing PCB contamination from the application of sludge to land.

CONCLUSIONS

1) The degradation of Aroclor 1254 in both the Celina and Brookston soils in 16 weeks ranged from 0.1 to 2.0 percent. Long-term build up of PCB's in soils can be expected.

2) Volatilization of Aroclor 1254 was higher from the Celina than the Brookston soil which may be related to differences in soil organic matter. Sewage sludge also reduced the volatilization of Aroclor 1254 from both experimental soils.

3) Higher concentrations of Aroclor (12.5 to 64.5 µg/20 g dry soil) may have inhibited the respiration rate of specific soil microorganisms capable of degrading PCB's in both experimental soils.

4) A maximum of 0.01 percent of applied Aroclor 1254 was taken up by Kentucky 31 fescue plants from sewage sludge amended Celina and Brookston soil. Even this small uptake of PCB by plants could be potentially serious due to biomagnification of PCB in the food chain.

5) After simulation of spray irrigation of ^{14}C-Aroclor 1254 amended sewage sludge to fescue leaf surface, about 6.2 percent of the PCB applied was actually absorbed by the leaf while 41.5 percent of that applied was present on the sewage sludge remained on the leaf surface. This concentration of PCB is many times greater than PCB taken up from soil and translocated to the plant tops.

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SECTION 8

SEWAGE SLUDGE LANDSPREADING IN OHIO COMMUNITIES: 1980 PERSPECTIVE

D. Lynn Forster, B.S., M.S., Ph.D.
The Ohio State University
Columbus, Ohio 43210

INTRODUCTION

The primary purpose of this report is to describe the extent of current practices in Ohio municipal sewage sludge landspreading as of 1980. The extent of landspreading and the characteristics of the sludge are summarized; sludge application rates and the resulting loadings of nutrients and metals to the soil are estimated; the crop acreage receiving sludge is projected, as well as the nutrient value to these crops; landspreading systems are described, and monetary costs of these systems are estimated. Finally, the relationships between landowners receiving the sludge and landspreading municipalities are described. Where possible, comparisons are made to landspreading practices that existed 5 years earlier. Ohio communities were surveyed in 1975 and 1980, and the survey results are the data base used for this analysis.

BACKGROUND

Landspreading in Ohio has a history as long as municipal waste treatment. But in the past decade interest in landspreading increased dramatically. First, there was more sludge for cities to dispose. Federal legislation and subsequent financial assistance caused communities to upgrade their wastewater treatment facilities, to remove more solids from effluents, and to produce more sludge. Second, cities faced a limited number of sludge disposal options, and for most cities, landspreading was the lowest cost alternative. Third, sludge contained plant nutrients which could be used on cropland as substitutes for nutrients from increasingly costly commercial fertilizer. With these incentives, landspreading sludge received increased support from community officials and farmers. However, some warned of potential problems with landspreading sewage sludge.

Concern was expressed that surface water quality might deteriorate due to runoff from landspreading fields, groundwater might receive excessive levels of chemicals from leaching, soils might be permanently damaged due to the accumulation of toxic materials, plants might take up and accumulate heavy metals which could be dangerous to plant growth and human health, viruses or other pathogens might create potential health problems, and nuisance odors might result. Extensive research throughout the United States has demonstrated that sludge may be a valuable product for agriculture and landspreading may be a practical disposal option, but also that improper management of sludge may produce pollutants injurious to soil, plants and human health (CAST, 1976; Information Transfer, 1978; Page, 1974).

In 1975, a survey of Ohio landspreading communities was conducted to identify the extent of landspreading and the current methods and practices used. Results indicated that numerous communities landspreading sludge were following less than satisfactory management programs. Sludge was being disposed, not judiciously landspread. Application rates were largely unknown, more than half the communities had no knowledge of the heavy metals content of their sludge, many did not know the nutrient content of the sludge, and most lacked information about the nutrient requirements of the cropland receiving the sludge. In short, landspreading was being conducted on a widespread basis, but the majority of communities were not using management practices which would assure practical, yet environmentally safe programs.

In 1977, the present project was initiated in Ohio to demonstrate landspreading practices which provided communities an economical method of sludge disposal, provided crop nutrients to farmers, and minimized health and environmental risks.

This section summarizes current (1980) Ohio landspreading practices. A survey of Ohio landspreading communities was used to gather this information. Where possible, comparisons are made to 1975 practices found in a similar survey. Changes in practices have occurred, of course. Many of these changes may be due to the educational program. However, not all changes can be attributed to this program. U.S. Environmental Protection Agency guidelines and regulations have had an effect on these practices. Also, economic conditions have encouraged the expansion of landspreading and have shaped practices being used.

SURVEY PROCEDURE

District offices of the Ohio Environmental Protection Agency provided the locations of treatment plants which were thought to be conducting landspreading programs. Eighty communities were identified, and a questionnaire was mailed to each of these communities. The questionnaire was to be returned to OFB offices. Another questionnaire was mailed to those communities not responding to the first one. From these two mailings, 63 communities (79%) returned completed questionnaires. A few of these communities reported that landspreading was not being used and incineration or landfilling was the principal method of disposal. Fifty-six communities completing the questionnaire were identified as landspreading and were used in this analysis.

CHARACTERISTICS OF TREATMENT PLANTS AND SLUDGES

The characteristics of treatment plants using landspreading are shown in Table 8.1, and the characteristics of the sludges from those plants are shown in Table 8.2. It is estimated that the total amount of sewage treated in Ohio is 1,373 million gallons per day (MGD) (Logan and Miller, 1980). Table 8.1 indicated the mean flow of the 56 treatment plants to be 6.14 million gallons per day. Thus, landspreading is the sludge disposal method used for at least 344 MGD (25%) of the total flow from all Ohio sewage treatment plants. Undoubtedly, other landspreading communities are not included in these survey results and thus landspreading in Ohio probably accounts for more than 30% of sludge disposal.

The mean amount of sludge produced from each of these landspreading communities is 1,243 dry metric tons per year. However, a few very large treatment plants skew this distribution. The median sludge production, 689 dry metric tons per year, provides a better representation of the amount of sludge from the typical plant. Most of this sludge is treated by anaerobic or aerobic digestion.

The characteristics of the sludges (Table 8.2) are similar to those found in other studies (Sommers, 1977; Tabatabai and Frankenberger, 1979). The total nitrogen can be divided into organic and ammonia forms. However, too few cities reported this division to provide meaningful results. Metal concentrations are within the range usually seen in the United States. One community in the sample has unusually high metal concentrations which increased the mean substantially. Again, the median concentration is a better reflection than the mean of typical concentrations in Ohio communities.

NUTRIENT AND METAL LOADINGS

For those communities reporting both a) nutrient and metal concentrations and b) sludge application rates, nutrient and metal loading rates were computed. The results are shown in Table 8.3. Again, a few communities with high sludge application rates produce a relatively high mean annual application rate of 17.0 dry metric tons per hectare. The median annual rate is 8.5 metric tons per hectare. Also, the mean nutrient and metal loadings are much higher than the median loadings due to a few high rates of sludge application.

Most communities are well within existing regulations and guidelines for metal loadings (Miller et al., 1979; EPA, 1979). The community with the most extreme metal loadings exceeds the maximum allowed annual cadmium loading. However, all other communities' metal loadings are low enough to prevent any plant toxicity or damage to animal or human health. It appears that Ohio communities are generally spreading sludge in an environmentally safe manner.

Nutrients are being applied at relatively high rates. Both the mean and median phosphorus rates (212 and 157 kg P/ha) supply more

TABLE 8.1. SLUDGE TREATMENT PLANT CHARACTERISTICS FOR 56 OHIO LANDSPREADING COMMUNITIES, 1980

Characteristic	Mean	Median	Number Reporting
Treated Flow (million gallons per day)	6.14	3.0	56
Sludge Production			
Dry metric tons per day	3.7	2.1	56
Dry metric tons per year	1,348	760	56
Sludge Treatment Method (%)			
Anaerobic	55.0		
Aerobic	38.3		
Lime	4.0		
Heat	1.7		
Other	1.0		

Source: Survey results.

TABLE 8.2. SLUDGE CHARACTERISTICS FOR 56 OHIO LANDSPREADING COMMUNITIES, 1980

Characteristic	Mean	Median	Number Reporting
Solids (%)	6.1	4.5	55
Plant Nutrients (%)			
Nitrogen--TKN	3.1	2.7	23
Phosphorus	1.9	1.9	24
Potassium	0.4	0.3	18
Metals (μg/g)			
Cadmium	49	12	33
Zinc	1,889	1,392	39
Copper	796	540	39
Nickel	304	95	38
Lead	697	250	30

Source: Survey results.

TABLE 8.3. ANNUAL SLUDGE APPLICATION RATES: NUTRIENT AND HEAVY METAL LOADINGS FOR 56 OHIO LANDSPREADING COMMUNITIES, 1980

	Mean	Median	Maximum	Number Reporting
	mt/ha			
Annual Sludge Application Rate	17.0	8.5	101	35
	kg/ha			
Nutrient Loadings				
Nitrogen--TKN	361	213	1,816	16
Phosphorus	215	157	535	18
Potassium	46	22	282	14
	kg/ha			
Metal Loadings				
Cadmium	0.7	0.2	6.8	22
Zinc	19.5	11.1	63.6	25
Copper	7.8	3.8	54.5	26
Nickel	2.2	1.1	11.4	24
Lead	7.8	3.0	60.3	19

Source: Survey results.

phosphorus than is typically required by a crop in one growing season in Ohio. Thus, some phosphorus from sludge is carried over to later growing seasons. Available nitrogen supplied by the mean and median annual loading rates is generally lower than the requirements of corn, but it approximates the requirements of small grains, hay, and pasture. Potassium loading rates are lower than the potassium requirements of most crops. In most cases, supplemental applications of potassium from other sources are required.

BENEFITS OF SLUDGE TO CROPLAND

Acreage receiving sludge very greatly between communities. In some communities, substantial row crop acres are landspread with low (4.5 mt/ha or less) application rates. In other communities, the receiving land remains idle during landspreading, and high application rates are used over a small number of acres. The crop acreage affected by landspreading in these 56 communities are:

	Hectares	Percent
Corn	4,550	44.8
Soybeans	1,480	14.5
Pasture and Hay	1,430	14.1
Idle	1,230	12.1
Small Grains	940	9.3
Other	530	5.2
	10,160	100.0

Thus, nearly 88% of the land receiving sludge is in crops during the year of sludge application. These crops utilize the nutrients in the sludge, and the nutrients substitute for commercial fertilizer.

The potential gross fertilizer value of sludge from these 56 communities is $2.3 million annually.[1] To realize all these benefits, communities must spread sludge at rates which supply nutrients in amounts which do not exceed crop requirements. With present application rates, phosphorus is supplied in excess of crop needs, and some benefits are lost in most communities.

LANDSPREADING SYSTEMS

About two-thirds of the surveyed communities own sludge application equipment and spread their own sludge. The other one-third contract landspreading services from commercial haulers. While contract haulers are used by communities of all sizes, they tend to be used by the larger landspreading communities. Communities using contract haulers produce an average of nearly 2,800 dry mt per year, while communities using their own equipment average only 829 dry mt per year. Contract haulers spread 65% of the sludge landspread in Ohio while community owned systems spread only 35%.

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[1] Each community spreads on average of 1,244 dry metric tons per year, or 69,644 dry metric tons are spread by all communities. The nutrient value of sludge is approximately $30 per dry metric ton.

From estimates provided by surveyed communities, landspreading cost functions were estimated. Each community provided the total operating costs (e.g., labor, fuel, maintenance, and contract hauler fees), was well as the capital investment in sludge disposal equipment. This capital investment is converted to annual fixed costs (i.e., depreciation and interest).[2] Total annual costs of landspreading (operating costs plus fixed costs) are divided by the amount of sludge landspread to arrive at the cost per dry ton. For communities contracting hauling services, annual costs are the fees paid by the community to the hauler.

Costs are related to the size of the community by the following:

$$\text{Cost per dry metric ton} = a_0 + a_1 \frac{1}{\text{Annual dry metric tons}} \qquad 1$$

Coefficients a_0 and a_1 are estimated using regression analyses for both community owned systems and contract hauler systems.[3] Results are shown in Table 8.4. Generally, the statistical estimates of Equation 1 are better for community owned systems than for contract hauler systems. The R^2 for the estimated relationship for community owned systems was much higher than that for contact hauler systems. Also, the regression coefficient relating cost per ton to community size (a_1) was more statistically significant for community owned systems. For most community sizes, landspreading costs for contract hauler systems are about three times higher than the costs for community owned systems (Table 8.5).

Using these estimates, sludge landspreading costs these Ohio communities nearly $7.8 million or an average of $111 per dry metric ton. As previously stated, about $33 per dry metric ton may be recovered as benefits from nutrients in the sludge. Of course, these benefits tend to be captured by farmers receiving sludge rather than the community producing the sludge.

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[2] Annual fixed costs are assumed to be equal to 40% of the capital investment (1980 dollars). Equipment life is assumed to be 3 years, and the interest is assumed to be 14% of the mid-life value.

[3] Several regression models were estimated. Generally, these models hypothesized that economies of size were present. Also, distance to landspreading site was incorporated in some of the models but was not found to be statistically significant.

TABLE 8.4. REGRESSION ANALYSIS RESULTS FOR OHIO COMMUNITY OWNED AND CONTRACT HAULER SYSTEMS, 1980

	Community Owned (N=24)	Contract Haulers (N=12)
I. Regression Equations		
a. Regression coefficients		
a_0 Intercept	33.79	99.13
a_1 $\frac{1}{\text{Annual dry tons}}$	17,664* (8.20)†	65,307‡ (1.66)
b. R^2	0.75	0.22
II. Annual Sludge Production		
Mean (dry metric tons)	670	2,237
Median (dry metric tons)	327	1,110
III. Cost per Dry Metric Ton at		
Mean annual production	$64	$139
Median annual production	$92	$168

* Statistically significant at <.01 level.

† Numbers in parentheses are the t-values for the regression coefficients.

‡ Statistically significant at <.12 level.

TABLE 8.5. LANDSPREADING COST ESTIMATES BY AMOUNT OF ANNUAL SLUDGE PRODUCTION, OHIO, 1980

Annual Sludge Production	Community Owned	Contract Haulers
dry metric tons	$/dry metric ton	
454	76	254
908	56	181
1,362	47	158
1,816	45	146

TESTING AND MONITORING

Most communities have adequate knowledge of the contents of their sludge (Table 8.6). The survey results indicate that 93% of the communities surveyed know the metals content of their sludge, and most of these communities also know the nutrient content. The remaining 7% of the communities have a minimal analysis which provided solids content, pH, and a few other characteristics.

These results contrast sharply with the situation 5 years earlier (Forster et al., 1976). Then only 42% had thorough knowledge of their sludge, and 21% of the communities had no sludge analysis. Generally, landspreading communities are also more knowledgeable of the soils receiving sludge than they were 5 years earlier. About half of the communities conduct soil testing programs prior to sludge application (Table 8.7). Five years earlier, very few had soils tested prior to application. About half of the communities also monitor the soils after sludge application, which is up sharply from 5 years earlier. Monitoring of plant tissue and water quality at the landspreading site is done by one-third of the communities, which is about the same as 5 years ago.[4]

EQUIPMENT

Most communities use tank trucks to spread sludge with a solids content of less than 10%. Of the 45 communities providing information about equipment, 41 were spreading liquid sludge and only 4 are spreading a dewatered sludge. Of the 45 communities, 19 have spreading vehicles with flotation tires. Flotation tires lengthen the period of time when vehicles have access to fields, and they reduce soil compaction. Another trend in equipment usage is the operation of separate "nurse" trucks to haul the sludge from the treatment plant to the disposal site. Of the 45 communities supplying equipment information, 15 are using nurse trucks.

LAND OWNERSHIP AND CONTRACTED ARRANGEMENTS

Most communities spread sludge on privately owned land (Table 8.8). A few pay the landowner a rental fee, a few receive payment for the sludge, but most communities use the landspreading site with no payments made by either party. A small proportion of the communities (11%) only use land owned by a governmental unit.

Most communities using privately owned land have an implicit understanding or oral agreement rather than a written contract. Written

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[4] Estimates of testing and monitoring programs tend to understate the extent of these programs. For example, communities hiring contract haulers may have no soil testing program; however, the hauler may be testing the soils.

TABLE 8.6. SLUDGE ANALYSIS PROGRAMS CONDUCTED BY OHIO COMMUNITIES, 1980

	1975 Survey		1980 Survey	
Type of Analysis	Number of Communities	Percent of Communities	Number of Communities	Percent of Communities
No analysis of sludge	9	21	0	0
Minimal analysis of sludge[*] (e.g., solids content, pH)	16	37	4	7
Thorough analysis of sludge[†]	18	42	51	93
nutrient content	(N.A.)		(36)[‡]	
metals content	(N.A.)		(51)	
Communities responding	43	100	55	100

[*] "Minimal" includes analyses for total solids content and volatile solids in the sludge.

[†] "Thorough" includes analyses for solids content, volatile solids, some primary nutrients (Nitrogen, phosphorus, potassium, and some heavy metals (cadmium, zinc, copper, nickel, boron, chromium, cobalt, manganese, mercury, molybdenum, lead).

[‡] Five of the communities not analyzing the nutrient content used contract haulers. It is likely that some of these haulers analyzed the sludge for nutrient content.

TABLE 8.7. SOIL TESTING AT LANDSPREADING SITE AND MONITORING OF LANDSPREADING SITE, OHIO LANDSPREADING COMMUNITIES, 1980

Type of Testing and Monitoring	1975 Survey Number of Communities	1975 Survey Percent of Communities	1980 Survey Number of Communities	1980 Survey Percent of Communities
Testing soil prior to application	4	9	27	49
Monitoring soils after application	8	19	24	44
Monitoring water quality near landspreading site after application	14	33	18	33
Monitoring plant tissue on landspreading site after application	18	42	18	33
Communities responding	43	100	55	100

TABLE 8.8. OWNERSHIP OF LANDSPREADING SITES

Owner	Number of Communities	Percent
Municipality or other governmental unit	6	11
Private	38	69
Some government owners and some private owners	11	20
Total	55	100

contracts are used by only 39% of the responding communities. Those that use written contracts typically include a clause specifying the application rate. Other commonly used clauses include: a) specification of the type and frequency of sludge analysis, b) specification of acceptable sludge quality, and c) restrictions on time of application.

CONCLUSIONS

The quality of landspreading programs in Ohio has improved substantially over the past 5 years. Communities are better aware of the contents of their sludge and spread it in a more judicious manner. Ohio communities appear to be landspreading sludge in a manner which provides a low cost disposal option, provides crop nutrients to farmers, and minimizes health and environmental risks.

The contents of Ohio sludges are typical of those seen throughout the United States. Application rates are moderate. Loadings of metals are generally well within EPA guidelines and regulations. These metal loading rates are low enough to prevent damage to soils, plant toxicity, or impairment of human or animal health. Phosphorus in the sludge is being applied at rates which exceed crop requirements and thus some carryover of phosphorus is occurring. However, phosphorus application rates are not high enough to affect surface or ground water. Communities are conducting testing programs which provide adequate information on the contents of their sludges.

A representative landspreading community has the following program. Anaerobically or aerobically digested liquid sludge (4.5% solids) is landspread by a tank truck on privately owned land within a few kilometers of the treatment plant. A verbal agreement is reached between the landowner and the community about application rates, time of application, and fields to receive the sludge. Payments are made by neither the landowner nor the community. About 690 dry metric tons are applied each year by the community at 8.5 dry metric tons per hectare. Land receiving the sludge is primarily cropland, with corn, soybeans, pasture, and hay being the predominant crops. The community is bearing costs of about $63 per dry metric ton if it does its own landspreading. If it contracts the spreading to a contract hauler, costs are subtantially higher. The community tests the sludge and has knowledge of its nutrient and metal content. Soil testing is performed in order to project crop nutrient requirements. Some monitoring of the landspreading site is done after landspreading.

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SECTION 9

ECONOMIC CONSIDERATIONS IN LANDSPREADING SEWAGE SLUDGE

D. Lynn Forster, B.S., M.S., Ph.D.
The Ohio State University
Columbus, Ohio 43210

In the past decade, the nation has increased its awareness of the finiteness of our natural resources and their ability to assimilate the by-products of our industrial society. Many of these by-products were discharged in the effluents from municipal wastewater treatment plants. Pollutants in effluents have been sharply curtailed over the past decade as a result of implementing provisions of the Federal Water Pollution Control Act Amendments of 1972 (P.L. 92-500, 18 Oct. 1972). As treatment plants have improved the quality of effluents, a new problem has been created: how to dispose of the increased quantity of treated solids (i.e. sludge) removed from the effluent. In 1970, 3.6 million metric tons of sludge were produced, and it is projected that over 7.3 million metric tons will be produced in 1985 (CAST, 1976).

The objectives of this section are to (a) summarize previous research comparing the costs of various sludge disposal methods, (b) outline alternative systems for one promising disposal method, landspreading, and (c) make economic comparisons of alternative landspreading systems.

SLUDGE DISPOSAL METHODS

Sludge is far from a uniform product. Its characteristics vary from community to community. These characteristics are determined, in part, by the wastewater treatment processes. Sludge can be stabilized by lime stabilization, anaerobic digestion, aerobic digestion, or thermal conditioning. It can be further treated by thickening processes or dewatering methods to increase the proportion of solids in the final product. Finally, it can be disposed of by either burning (incineration), composting, landfilling, or landspreading.

Sludge treatment and disposal options are described in detail in numerous publications (e.g. EPA, 1975, 1978; REA, 1978). These options are only briefly described here.

Sewage sludge incineration has been practiced for several decades. Cheap energy and minimal or nonexistant air pollution control encouraged its adoption as a practical and inexpensive method of reducing sludge volume. Incineration typically is preceded by processes to reduce the water content of the sludge. For example, sludges might be thickened, digested, and dewatered, or they might be stabilized chemically and dewatered before entering the incineration process. Although the heat value of a dry ton of sludge is high, the water content of most sludges requires an auxiliary fuel source to maintain combustion. Of course, rising fuel costs are the major drawback to this system.

Due to rising fuel costs, partial pyrolysis has been demonstrated to be a means of combusting sludge without large amounts of supplemental fuel. The principle is to reduce the amount of air heated to combustion temperature which prevents wasting energy to heat excess air in the furnace. Pilot operations have shown advantages of slightly lower operating costs and reduced air emissions compared to traditional incineration processes.

Cocombustion is another method to reduce the fossil fuel requirements of incineration. Sewage sludge is combined with any number of materials and then burned. A potential advantage is that a waste material, such as municipal solid waste, can be disposed while providing an autogenous sludge feed (EPA, 1978). Besides handling both solid waste and sludge in an environmentally acceptable manner, the process produces heat, may provide benefits as an energy source, and may slightly reduce operating costs.

Composting is another sludge disposal option. Usually dewatered sludge is mixed with a bulking agent (e.g. wood chips) to reduce moisture content. Piles of the mixture are constructed and aerated for 21 to 30 days. Piles are dismantled and allowed to cure for another 30 days. The compost is then screened to recover the bulking agent and the stabilized sludge is landspread or landfilled. Composting may be a viable alternative for many locations, but the basic processes are still in the development and demonstration phase.

Lagooning involves dumping sludge into a large open pit. The liquid is decanted off, and the sludge is allowed to dry. When the lagoon is full, it is covered by a layer of earth, and another lagoon is started. Two potential problems are present. First, the lagoon floor may be permeable and permit leaching, and second, odors may produce adverse public reaction. But more importantly, lagooning must be viewed as only a temporary disposal method due to the land constraints facing most communities.

With landfilling, dewatered sludges are buried in a trench or area landfill. The sludge is periodically covered with a layer of soil to control odor. Sludges placed in area landfills may be mixed with soil in order to support equipment working on top of the landfill. Sludge may also be mixed with solid waste and codisposed in landfills. Sites must be selected which prevent pollution of surface or ground waters. In addition, odors must be controlled.

Landspreading, the focus of this report, utilizes sludge treated by aerobic or anaerobic digestion. Before landspreading, stabilized sludge may undergo dewatering to reduce its volume. Methods of handling and application are quite diverse. Tank trucks or tank wagons generally are used to haul liquid sludges with 1 to 7 percent solids. Truck spreaders are used for dewatered sludges with solids content of 15 to 50 percent. Irrigation of liquid sludge is possible. Also, rail, barge, or pipeline transportation systems could be used.

Another treatment method is land treatment of both effluents and sludges. It is based on the use of soil and its biological systems as a treatment process. Primary or secondary treatment processes may be followed by land treatment. The result is that up to 100 percent of BOD, suspended solids, nitrogen, and phosphorus can be removed from the wastewaters before final discharge into water bodies.

ECONOMIC COMPARISONS FOR SLUDGE DISPOSAL METHODS

A number of researchers have investigated the costs of alternative sludge disposal methods. Burd (1968) reviewed data available in the late 1960s and drew some generalizations about relative costs of alternative sludge disposal methods. Estimates were that capital and operating costs were $17 per dry metric ton for landspreading liquid sludge and $28 per dry metric ton for landspreading dewatered sludge. Landfilling dewatered sludge was estimated at $28 per dry metric ton, and incineration at $33 to $46 per dry metric ton. Due to a lack of data, Burd was unable to relate these costs to volume of sludge produced by the plant. A weakness of Burd's analysis was that no economic benefits were attributed to the plant nutrient value of landspread sludge.

Ewing and Dick (1970) compared the relative costs of the principal disposal methods. Their estimates showed landspreading liquid sludge costing $17 per dry metric ton, landspreading dewatered sludge $28 per dry metric ton, lagooning $20 per dry metric ton, and incineration $55 per dry ton. Again, no benefits were attributed to landspreading. However, landspreading and incineration costs were compared for a range of community sizes, and landspreading costs were approximately $44 per dry metric ton less than incineration costs over a wide range of community size.

More recent estimates by Shea and Stockton (1975) again found landspreading as the least expensive method of sludge disposal. Their estimates included the costs of thickening and disgestion as well as costs for ultimate disposal (i.e. landfilling, landspreading, and incineration). Table 9.1 shows the relative advantage of landspreading over a range of treatment plant size.

Shea and Stockton's landspreading costs were based on the assumption that land was purchased for spreading sites. This assumption biased landspreading costs upward since most landspreading communities spread sludge on land owned by individuals. They pay no rent for the

TABLE 9.1. COSTS OF SLUDGE PROCESSING AND DISPOSAL, BY DISPOSAL METHOD AND TREATMENT PLANT SIZE

Plant Size		Disposal and Processing Method		
MGD Flow	Sludge (dry metric t/yr)	Vacuum Filter Incinerate, Truck Landfill	Digestion, Truck, Landspread	Digestion, Truck, Landfill
		---------------- $ per dry metric ton	----------------	----------------
2	490	411	230	383
3	735	323	213	294
5	1224	257	194	235
10	2449	191	162	176
15	3673	162	147	147

Source: Adapted from Shea and Stockton (1975).

land nor do they pay any land ownership costs as Shea and Stockton's analysis assumed. Also, their analysis attributed no benefits to the plant nutrients provided by landspreading.

Colacicco et al. (1979) provided estimates of sludge disposal costs and a summary is shown in Table 9.2. Again, landspreading was shown to be an economically advantageous method of sludge disposal.

Land treatment of wastewater appears to be a promising treatment technology for small communities, for areas where water is in short supply, or for those communities where removal of nearly all pollutants from the effluent is required. Capital and operating costs may be lower than with conventional treatment and sludge disposal systems. Young and Carlson (1974) found that land treatment reduced costs compared to conventional treatment and sludge disposal systems. They projected savings of $0.11 per 1000 liters of wastewater for the 0.5 MGD plant and $0.04 per 1000 liters for the 10 MGD plant. Williams et al. (1977) compared land treatment and conventional treatment systems in a number of small Michigan communities. Land treatment systems had lower initial capital outlays and annual operating costs than did the conventional treatment systems. However, it is concluded in Young and Epp (1978) that acreage requirements for wastewater treatment suggest that land application is most applicable to small communities or for treatment of only part of the total wastewater from a large community.

BENEFITS OF LANDSPREADING SLUDGE

The primary benefit of sludge is its nutrient value. Nitrogen, phosphorus, and potassium concentrations average about 3.3, 2.4, and 0.3 percent, respectively, of dry sludge. These nutrients are required by most plants, and applications of commercial fertilizers are used with growing crops to supply sufficient quantities of these nutrients. Sludge can provide at least part of these nutrients. At the recommended sludge application rates (4.5 to 7 dry metric tons per hectare), sludge supplies at least part of the nitrogen and frequently all of the phosphorus needed for growing crops.

There may be some benefits for sludge as a soil conditioner on cropland. Organic matter in the soil enhances soil texture, promotes aeration and increases moisture-holding capacity. All of these characteristics may lead to increased crop production. If soils have been "run down" to the point where organic matter content is low, then application of sludge could have a significant effect. If, on the other hand, the soil has been well-managed prior to sludge application, little effect may occur. Similarly, in years with good rainfall, the moisture retention effect may not be significant while in dry years it may be important. With this uncertainty relating to the value of sludge as a soil conditioner, one may either assume no difference or make some arbitrary adjustment to represent the effect over a period of years. Typically, sludge at recommended application rates provides such small benefits as a soil conditioner for cropland that it can be ignored.

TABLE 9.2. COMPARATIVE COSTS FOR VARIOUS SLUDGE DISPOSAL PROCESSES (1976 DOLLARS)*

Item	Range of Costs (Dollars Per Dry Metric Ton)
Digested sludges:	
Ocean outfall	11 to 39
Liquid landspreading	22 to 60
Digested and dewatered sludges:	
Ocean barging	34 to 49
Landfilling	25 to 58
Landspreading	29 to 106
Dewatered sludges:	
Trenching†	128 to 148
Incineration†‡	63 to 103
Heat drying†	68 to 127
Composting† ‡	39 to 55

* Source: Colacicco et al. (1977).

† Costs exclude transportation of sludge to site.

‡ Costs exclude cost of removal of residues and benefits from resource recovery.

Most sludges have many of the micronutrients that are needed by crops. However, some of the micronutrients in large quantities can be detrimental to the crop. The metal content of some sludges makes them unfit for use on land. Another problem with many sludges, especially dewatered sludges, is that they may have a high salt content. These salts are easily leachable, but can create problems when applied in large quantities in arid regions.

There is a large non-farm demand for good quality topsoil and soil conditioners that sludge products have helped fill. Sludge has been successfully used in reclaiming surface mines. Sludge has been used to renovate urban park land and has saved hundreds of thousands of dollars in topsoil costs. Sludge and sludge products have been found to compare successfully with potting mixes for nursery applications. Likewise, sludge-derived products have been sold to homeowners as soil conditioners.

The benefits depend on the use of the sludge, the soil characteristics, the nutrient content of the sludge, the application rate, and the price of other nutrient sources which sludge is replacing. For use on cropland, the potential value of sludge may total about $30 per dry metric ton as shown in Table 9.3.

To realize all the potential value of sludge, the recipient must restrict sludge application to relatively low rates. Application rates in excess of 4.5-7 metric tons per hectare annually result in much of sludge nutrients being unused by the crop. These unused nutrients are either lost for crop growth, or their use by crops is delayed until later growing seasons. The appropriate sludge application rate for a particular site is governed largely by the type of crop being grown, the yield goal for that crop, the existing nutrient level of the soils at the spreading site, and the nutrient content of the sludge. Local agricultural experts need to be consulted to determine the nutrient needs of the crop. Treatment plant officials then should determine the amount of nutrients available in its sludge. Information about crop nutrient needs should be compared to the supply of nutrients in the sludge to determine the proper application rate. Supplemental application of commercial fertilizer likely would be required to meet any nutrient deficiencies.

OUTLINE OF ALTERNATIVE SLUDGE LANDSPREADING TECHNOLOGIES

Before landspreading, the stabilized sludge may undergo further dewatering treatment to reduce its volume. Sludge can be dewatered by chemicals, mechanical processes, heating, drying, or some combination of these four processes. Solids content before dewatering typically ranges from 1 to 7 percent, but after dewatering solids range between 15 and 50 percent.

Methods of handling and applying sludge during land application are quite diverse. The most typical method is the use of tank trucks or tank wagons to haul and spread sludge having 1 to 7 percent solids.

TABLE 9.3. POTENTIAL VALUE OF NUTRIENTS IN ONE DRY TON OF SEWAGE SLUDGE*

	Percent of Dry Sludge	Value ($/metric ton)
Nitrogen†	3.3	$ 9.66
Phosphate (P_2O_5)	5.3	25.71
Potash (K_2O)	0.4	0.88
Total		$36.25

* Nutrient price assumptions: Nitrogen, $0.55 per kg; P_2O_5, $0.48 per kg; K_2O, $0.22 per kg.

† Nitrogen is assumed to be composed of 67 percent organic nitrogen and 33 percent ammonia nitrogen. This composition varies greatly between waste treatment plants. All ammonia nitrogen is available to the crop while only about 30 percent of the organic nitrogen is available.

These tank trucks or wagons may have high flotation tires for traversing soft ground and to minimize soil compaction problems. Attachments allow the liquid to be: (a) spread on the surface by gravity discharge; (b) spread on the surface to the side of the vehicle by pumped discharge; or (c) injected into the soil.

Truck spreaders may be used when dewatered sludge is spread. This semi-solid sludge may be hauled and spread by a conventional box spreader which is ordinarily used to field spread animal wastes. Truck spreaders also are available which allow surface spreading. Direct incorporation into the soil may be accomplished by using a plow, disc, or injection equipment.

Sprinkler irrigation or overland flow irrigation are other possible sludge disposal techniques. These systems for sludge disposal also may be used for tertiary treatment of effluent. With the sprinkler irrigation system, the liquid is sprayed on the land by either a solid-set system or a self-propelled system. Aerosol drift may present problems as more human contact with pathogens is possible. The overland flow system allows sludge to be discharged at the top of a slope and flow to the remaining acreage. A variation of this method, ridge and furrow irrigation, can be used with row crops.

Storage may be part of a landspreading system. It allows more timely applications for sludge to crops but, more importantly, provides an "escape valve" for sludge during the periods when adverse weather prevents landspreading. A lagoon for liquid sludge or a semi-solid storage installation may be located either at the treatment plant or at the landspreading site.

Transportation to the spreading site may be by the spreading vehicle or by separate transportation methods. For example, a large truck could be used to haul dewatered sludge to a spreading site where the sludge would be stockpiled for later application, or a large tank truck could be used to haul liquid sludge to a disposal site where the sludge could be pumped into a spreading vehicle or into temporary storage for later spreading.

LANDSPREADING COSTS

There are three main determinants of sludge landspreading costs: type of sludge disposal technology, the distance between the treatment plant and the landspreading site, and the volume of sludge. The following analysis compares costs of sludge disposal by volume of sludge and by disposal technology. Distance to landspreading site is included as an endogenous variable in the analysis. That is, it is assumed that 5 percent of the land in the community is available for landspreading, and each available parcel of land received 4.5 dry metric tons per hectare. Thus, the analysis assumes that the amount of sludge determines the distance to spreading sites.

Sludge landspreading costs have been made for five alternative technologies:

a) tank wagon hauling and spreading liquid sludge (5% solids),
b) tank truck hauling and spreading liquid sludge (5% solids),
c) truck spreader hauling and spreading dewatered sludge (25% solids),
d) a separate hauling unit transporting liquid sludge to the spreading site where it is spread by a tank truck (5% solids), and
e) a separate hauling unit transporting dewatered sludge to the spreading site where it is spread by a truck spreader (25% solids).

Assumptions about the values of cost parameters are shown in Table 9.4. Variable costs are estimated by multiplying the hourly variable cost charges by the time requirement shown in Table 9.5. Time requirements are a function of hauling and spreading technology. Those technologies spreading liquid sludge are causing substantial volumes of water to be handled. Therefore, those technologies using dewatered sludge have much smaller time requirements per dry ton than the technologies using liquid sludge.

Dewatering costs are included in the cost estimates for those technologies spreading sludge having 25 percent solids content. Vacuum filtration is assumed to be the method used to dewater the sludge. Vacuum filtration requires a high capital outlay and large annual fixed costs. Recent EPA cost data was used in estimating dewatering costs. These costs are assumed to be a function of treatment plant size. Dewatering costs range from $82 per dry metric ton for the very small treatment plant to $27 per dry metric ton for the treatment plant with volumes over 5000 dry tons per year (Anderson, 1977).

Using the preceding cost estimation assumptions, the following analysis compares costs per dry ton for the five landspreading technologies over a range of sludge volumes. Figures 9.1 through 9.5 plot the costs per dry ton as a function of the amount of sludge spread each year. In Figure 9.1, costs for relatively small wastewater treatment plants (180 to 900 dry metric tons per year) are analyzed. For these treatment plants, the tank wagon and tank truck technologies are clearly preferable. Large per unit fixed costs for technologies using separate hauling units or dewatering make these technologies high cost options.

As sludge volumes become larger (900 to 2700 dry metric tons per year), the tank truck technology spreading liquid sludge remains the low cost option (Figure 9.2). With volumes of 2700 to 4500 dry metric tons per year (Figure 9.3), spreading liquid sludge (5 percent solids) remains lower cost than spreading dewatered sludge, but using a separate hauling unit is a low cost option. Between 5400 and 9000 dry metric tons per year (Figure 9.4), costs are nearly the same for two technologies--the truck spreader using dewatered sludge and the tank spreader using liquid sludge transported by a separate hauling unit. For large sludge volumes (Figure 9.5), spreading dewatered sludge and using a separate haul vehicle is the low cost technique.

TABLE 9.4. COST ASSUMPTIONS FOR THE ALTERNATIVE TECHNOLOGIES

Technology*	Purchase Price ($)	Annual Fixed Cost† ($/Year)	Variable Cost‡ ($/Hour)
a) Tank wagon	42,000	16,800	16.49
b) Tank truck	56,000	22,400	15.47
c) Truck spreader	56,000	22,400	15.47
d) Hauling unit	75,000	30,000	18.88
& tank truck	56,000	22,400	15.47
e) Hauling unit	75,000	30,000	18.88
& tank truck	56,000	22,400	15.47
& loader, etc.	37,500	15,000	16.49

* Capacity of the tank wagon is 7,600 liters and it is pulled by a 100+ horsepower tractor; capacity of the tank truck is 6080 liters; capacity of the truck spreader is 6.3 metric tons; capacity of the hauling units are 22,800 liters of liquid sludge and 21.8 metric tons of dewatered sludge.

† Fixed costs are 40 percent of the purchase price. They include depreciation, interest, insurance, and maintenance.

‡ Variable costs include labor ($6.90 per hour) and fuel ($0.22 per liter).

TABLE 9.5. TIME REQUIREMENTS FOR ALTERNATIVE LANDSPREADING TECHNOLOGIES

Technology	Function	
	Transport (hours/metric ton/km)	Load & Unload (hours/metric ton)
a. Tank wagon	0.183	1.10
b. Tank truck	0.078	0.92
c. Truck spreader	0.017	0.19
d. Hauling unit	0.017	0.08
& tank truck		0.92
e. Hauling unit	0.004	0.03
& truck spreader		0.19
& loader, etc.		0.06

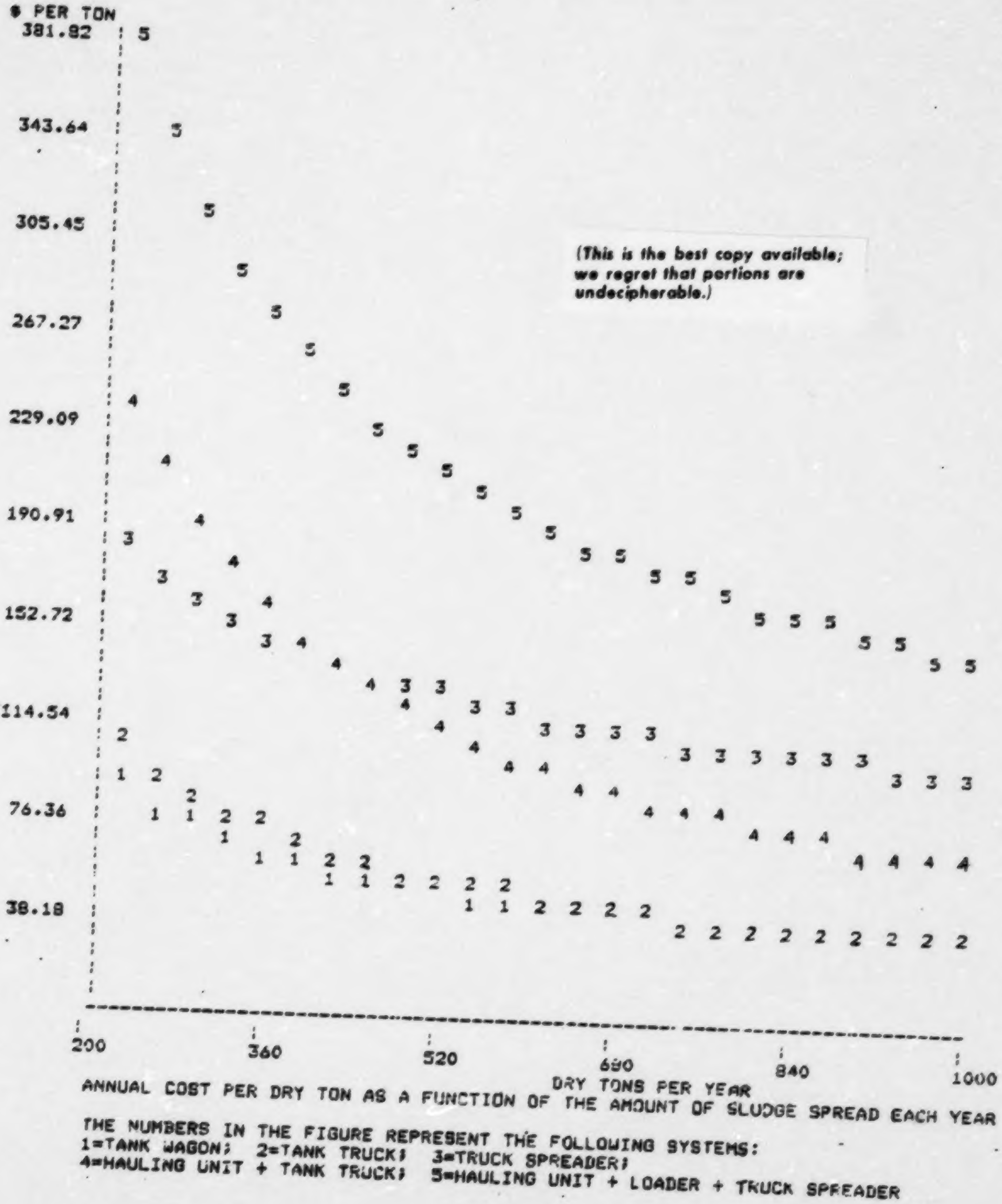

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Figure 9.1. **Computed costs of hauling and spreading sludge for communities producing 200-1000 dry tons per year.**

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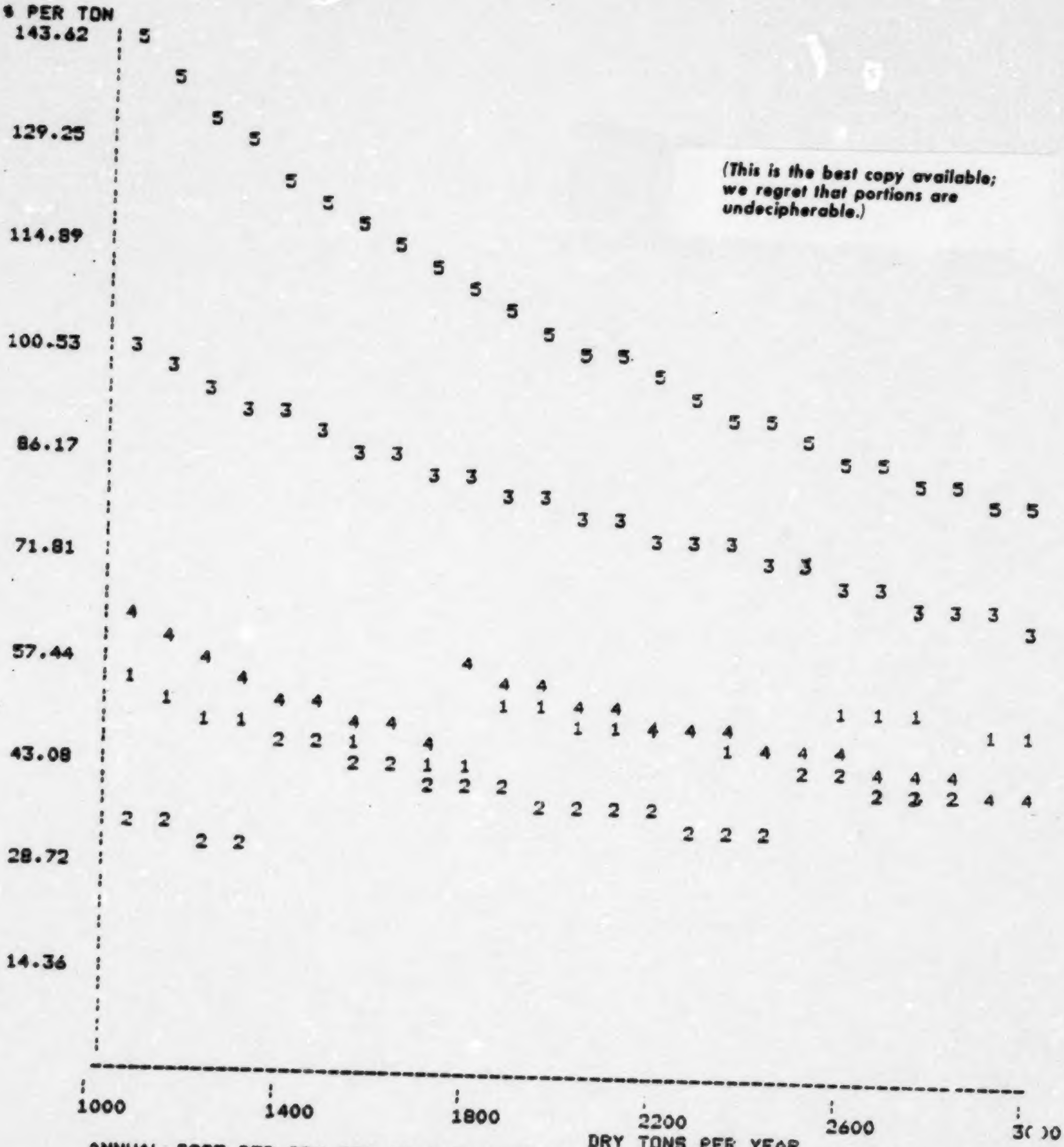

ANNUAL COST PER DRY TON AS A FUNCTION OF THE AMOUNT OF SLUDGE SPREAD EACH YEAR

THE NUMBERS IN THE FIGURE REPRESENT THE FOLLOWING SYSTEMS:
1=TANK WAGON; 2=TANK TRUCK; 3=TRUCK SPREADER;
4=HAULING UNIT + TANK TRUCK; 5=HAULING UNIT + LOADER + TRUCK SPREADER

Figure 9.2. Computed costs of hauling and spreading sludge for communities producing 1000-3000 dry tons per year.

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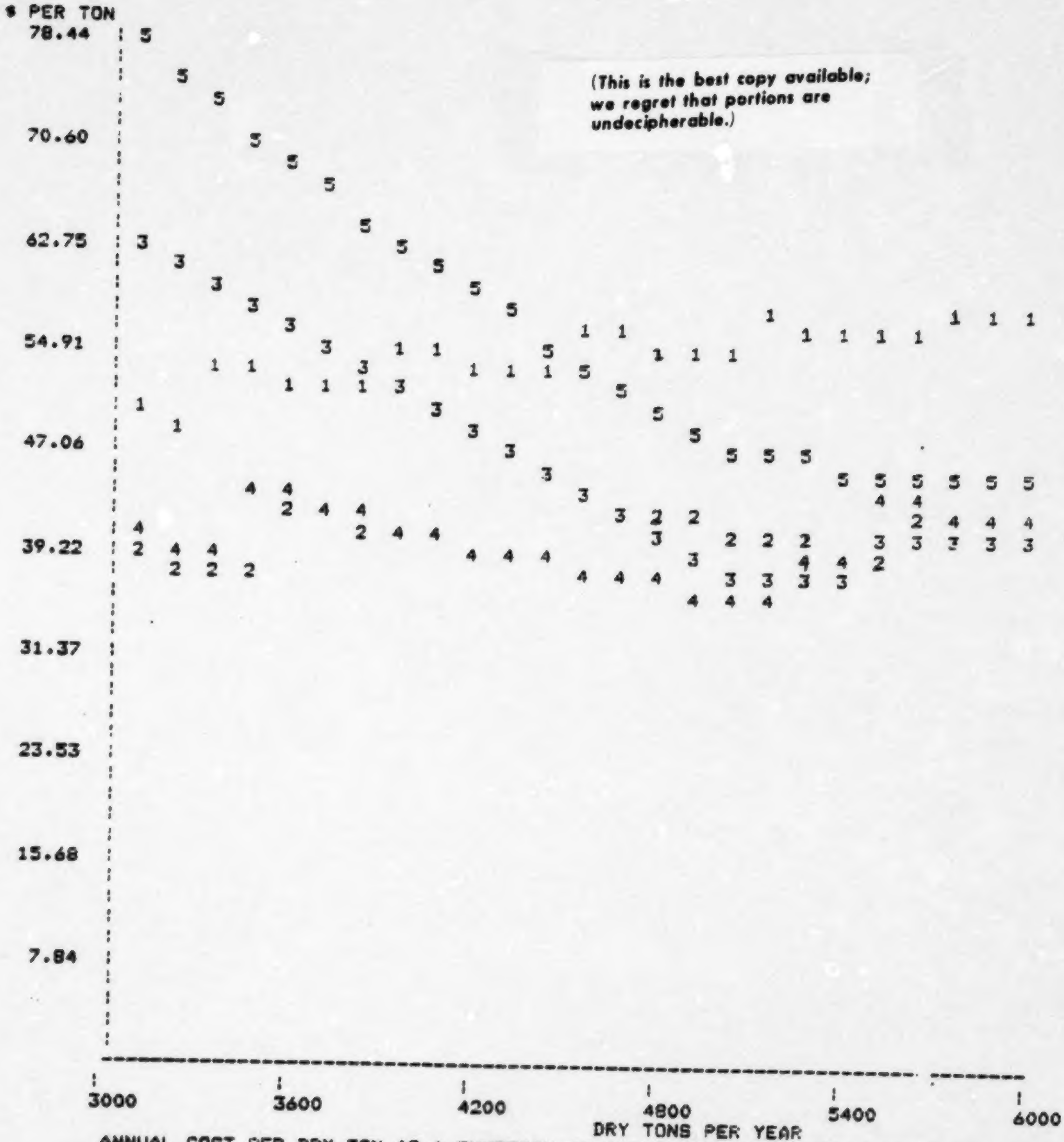

ANNUAL COST PER DRY TON AS A FUNCTION OF THE AMOUNT OF SLUDGE SPREAD EACH YEAR

THE NUMBERS IN THE FIGURE REPRESENT THE FOLLOWING SYSTEMS:
1=TANK WAGON; 2=TANK TRUCK; 3=TRUCK SPREADER;
4=HAULING UNIT + TANK TRUCK; 5=HAULING UNIT + LOADER + TRUCK SPREADER

Figure 9.3. Computed costs of hauling and spreading sludge for communities producing 3000-6000 dry tons per year.

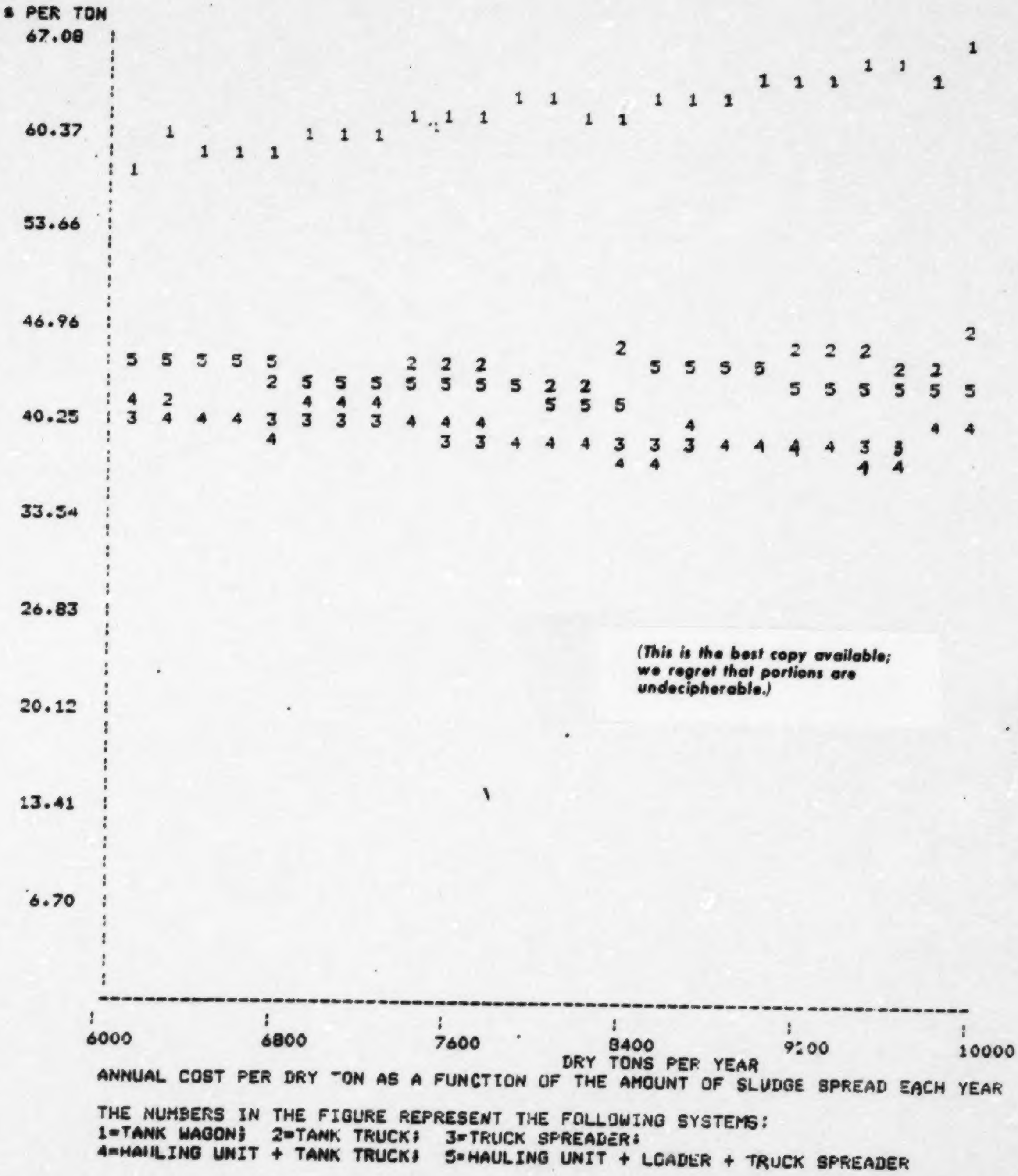

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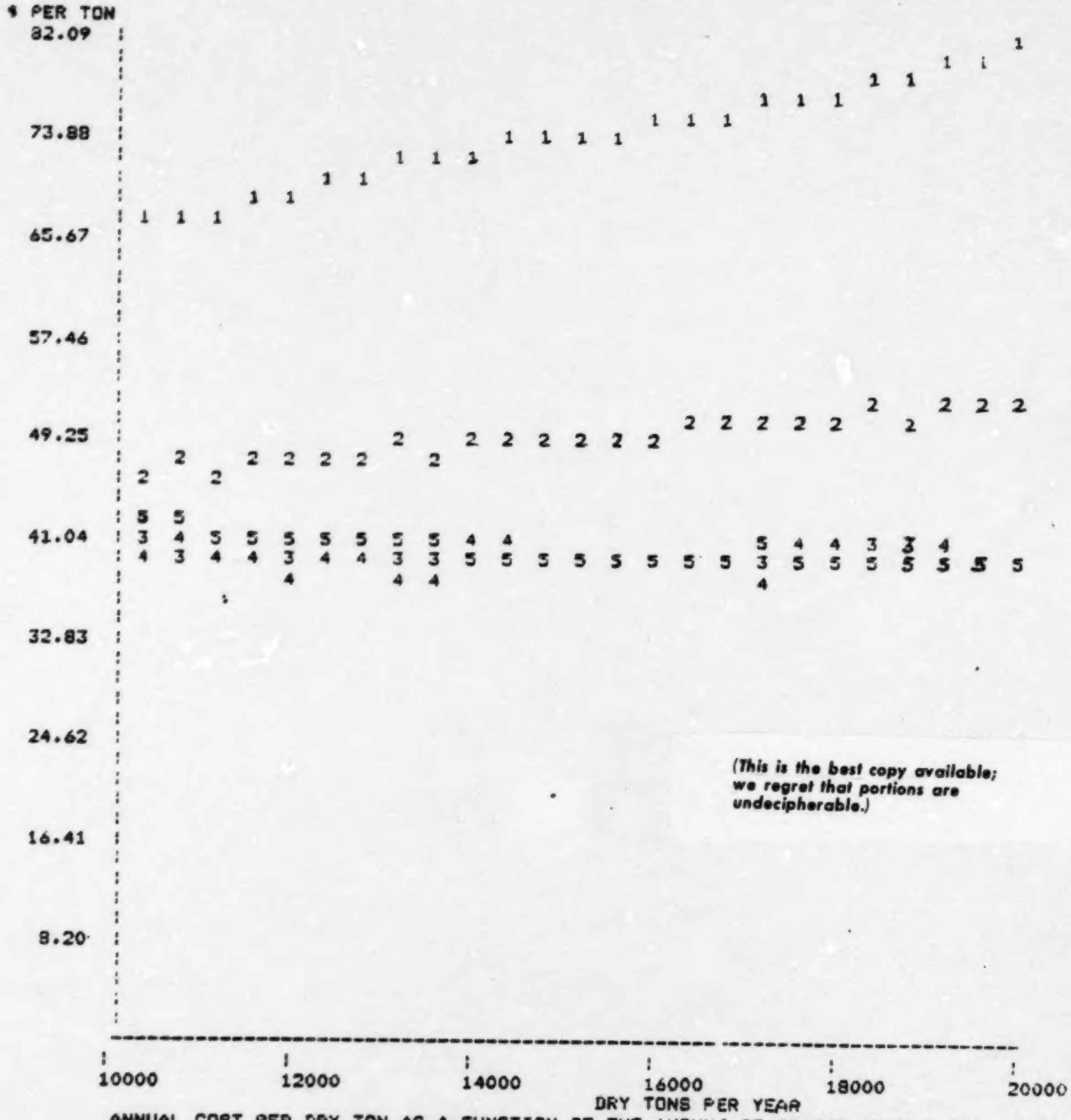
Figure 9.4. Computed costs of hauling and spreading sludge for communities producing 6000-10000 dry tons per year.



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ANNUAL COST PER DRY TON AS A FUNCTION OF THE AMOUNT OF SLUDGE SPREAD EACH YEAR

THE NUMBERS IN THE FIGURE REPRESENT THE FOLLOWING SYSTEMS:
1=TANK WAGON;  2=TANK TRUCK;  3=TRUCK SPREADER;
4=HAULING UNIT + TANK TRUCK;  5=HAULING UNIT + LOADER + TRUCK SPREADER
```

Figure 9.5. Computed costs of hauling and spreading sludge for communities producing 10000-20000 dry tons per year.

CONCLUSIONS

Landspreading is an economical method of sludge disposal for most communities. Generally, costs of landspreading are lower than costs of other disposal options such as incineration or landfilling.

Landowners may receive substantial benefits from landspreading. Sludge may provide many of the essential nutrients for plant growth. On cropland, benefits of sludge may total $67 per hectare if it is applied at low application rates. At the same time, there are some intangible costs to the landowner. The risks associated with pathogens and heavy metals are nearly nonexistent under a well managed landspreading system; nevertheless, these risks are present to some degree for all recipients of sludge. Similarly, recipients of sludge often incur some costs in answering neighbors' concerns and/or promoting landspreading in the community. Finally, in our society there is always the risk of legal action being brought against the recipient and the municipality by a third party.

The low cost landspreading technology is largely a function of sludge volume and distance to spreading site. In communities with large amounts of sludge and distant landspreading sites, dewatering sludge to 20 to 30 percent solids results in the lowest cost alternative. For most small and moderate size communities with nearby landspreading sites, spreading liquid sludge is preferred. Temporary storage is suggested for those periods when landspreading is not possible.

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SECTION 10

EFFECT OF SOIL PH ON THE EXTRACTABILITY OF CADMIUM APPLIED TO DIFFERENT SOILS WITH DIFFERENT SLUDGES

Randall E. James, B.S., M.S.
Robert H. Miller, B.S., M.S., Ph.D.
Terry J. Logan, B.S., M.S., Ph.D.
The Ohio State University
Columbus, Ohio 43210

INTRODUCTION

The objective of this study was to determine the effect of soil pH on the electrolyte extractability of cadmium when applied to soil with different types of sludges. Soil pH was adjusted by liming, and final pH was a function of limed and unlimed pH as modified by the pH buffer capacity of the different sludges.

METHODS AND MATERIALS

Soils

Six different soils were used in this study. The individual soil samples were collected from various locations within the State of Ohio. The soils include: a Bennington silt loam obtained from Fairfield County, a Kokomo (Brookston) silty clay loam from Franklin County, a Hoytville clay loam from Sandusky County, a Mahoning silt loam from Trumbull County, a Miamian silt loam from Franklin County, and a Spinks fine sand from Sandusky County. These soils were chosen because they were fairly representative of the major soil associations in the state.

Bulk soil (0-15 cm) samples were collected at field moisture levels and passed through a 2 mm sieve. All of the bulk samples were then allowed to partially air dry and stored at room temperature prior to beginning the experiments.

Additional information on each of the soils used in this study are presented in Table 10.1.

Sewage Sludges

Sewage sludges from five different waste water treatment plants in Ohio were utilized in this study.

TABLE 10.1. SELECTED CHARACTERISTICS OF SURFACE PLOW LAYER (0-15 CM) OF SIX OHIO SOILS

Soils	Classification	pH	Sand*	Silt*	Clay*
				%	
Bennington silt loam	Aeric Ochraqualfs	5.3	10.5	76.9	12.6
Kokomo silty clay loam	Typic Argiaquolls	6.1	9.7	53.6	36.7
Hoytville clay loam	Mullic Ochraqualfs	6.5	22.7	41.7	35.6
Mahoning silt loam	Aeric Ochraqualfs	5.6	23.3	64.3	12.4
Miamian silt loam	Typic Hapludalfs	6.3	21.4	56.5	22.1
Spinks fine sand	Psammentic Hapludalfs	6.5	92.0	4.5	3.5

* Approximate values obtained from the Soil Characterization Laboratory, The Ohio State University, Columbus, Ohio.

These sewage sludges were chosen to represent a wide variety of chemical and physical properties. Each sludge also represents a different sewage treatment process. It was felt that the different sewage treatment processes may have a bearing on how the sludges would react in soil systems.

The treatment processes used and the physical form of each sludge used in this study are:

* A lime stabilized liquid sludge from the City of Ashland.
* An anaerobically digested sludge cake from the Jackson Pike Treatment Plant in the City of Columbus.
* An aerobically digested (extended aeration process) liquid sludge from a Medina County Treatment Plant.
* An anaerobically digested sludge from the City of Springfield.
* An anaerobically digested lime treated sludge cake from Toledo, Ohio.

It is important to note that in the Toledo sludge lime is used as an aid to sludge thickening; while in the Ashland sludge it is used to kill pathogens, or stabilize the sludge. Therefore, more lime is needed to treat the Ashland sludge than is needed to treat the Toledo sludge. The results of laboratory analysis of each sludge is presented in Table 10.2.

Table 10.2 shows that the pH of the lime stabilized Ashland sludge is higher than the pH of the other four sewage sludges. The cadmium concentration of the Columbus sludge is two times that of any other sludge, and over ten times the concentration of the Medina sludge.

Liming Procedures

A subsample from the Bennington silt loam soil and a subsample from the Mahoning silt loam soil were each amended with 0.67g $Ca(OH)_2$ per kg soil and 0.87g $Ca(OH)_2$ per kg soil respectively, to raise the soil pH to approximately 6.5. The approximate $Ca(OH)_2$ application rates were determined using the Shoemaker, McLean, Pratt buffer procedure and the lime test index (Trierweiler, 1972). The limed samples were then leached twice with distilled water to remove any unreacted lime from the soils. After leaching was completed, the pH of these soils was again evaluated to insure that the soil pH had remained at approximately 6.5. The original six soils plus the two lime amended soils constituted a total of eight soil samples that were used for all of the experiments of this study.

Treatments

Subsamples (2270 g) of each of the eight soil samples were amended with the equivalent of 5 g dry sludge/kg soil or 11 dry metric tons/hectare of each sludge, plus a control soil sample to which no sludge was added. This made a total of 48 samples.

TABLE 10.2. CHARACTERISTICS OF SEWAGE SLUDGES FROM FIVE OHIO SEWAGE TREATMENT PLANTS

	Ashland Lime Stabilized	Columbus Anaerobically Digested	Medina Aerobically Digested	Springfield Anaerobically Digested	Toledo Anaerobically Digested
pH	12.3	8.2	6.7	7.4	7.8
Solids (%)	4.9	17.6	1.8	7.2	15.4
Cd (mg/kg)	24.6	57	3.6	38	11.8
Total P (mg/kg)	11051	23265	31255	14100	18800
TKN (mg/kg)	28470	38312	47396	29486	13070
K (mg/kg)	3541	3385	10314	4561	2878
Zn (mg/kg)	41306	5083	455	5493	2063
Ni (mg/kg)	22.6	454	30.6	291	235
Cu (mg/kg)	216	523	272	655	340
Pb (mg/kg)	1171	685	88	1161	392

An attempt was then made to adjust each sample to approximately 1/3 bar moisture content with distilled water. However, due to an oversight, it was found that in some, but not all cases, the amount of liquid added with the sludges with low solids content raised the soils to a level above 1/3 bar moisture content.

Each of the 48 samples were then subdivided into six replicate subsamples equal to 250 ml [illegible] samples and transferred to Erlenmeyer flasks, with 50 g of sludge/soil mixture in each flask. This made a total of 288 subsamples. The subsamples were incubated at room temperature (approximately 25 C). Each flask was opened and exposed to the air weekly to maintain aerobic conditions. Since the moisture content of the sludge/soil mixtures were not uniform due to the above-mentioned oversight, no attempt was made to maintain a uniform moisture content during the incubation period.

Incubation Period

After 0, 6, 12, 18, 27, and 85 days incubation, pH was measured on the individual subsamples of each sludge/soil mixture.

After 0, 6, 27, and 85 days, the sludge/soil mixtures were analyzed for 0.01 M $CaCl_2$ extractable Cd. Thus, 48 flasks were analyzed at the end of each incubation period. The 85 day incubation period was chosen because it represents the major part of one growing season. The incubation period began (Day 0) immediately after the soils were amended with sewage sludge.

Determination of Soil pH

At the end of each incubation period, four subsamples were removed from each flask after thorough mixing. Two of the samples were used to measure pH using a 1:1 soil:water ratio. The pH was determined in the remaining two samples using a 2:1 ratio of 0.01 M $CaCl_2$ to the soil/sludge mixture. All mixtures were stirred intermittently for approximately one hour and then read on a pH meter, using a glass electrode.

The pH determinations that used $CaCl_2$ were consistently lower than the water pH determinations. This could be predicted since the $CaCl_2$ would replace H+ ions on the soil exchange sites and increase the hydrogen ion concentration of the soil water, and thus produce a lower pH reading.

All of the pH data reported is the mean of two water pH determinations unless otherwise specified.

Extractable Cadmium Determinations

At the end of the 0, 6, 27, and 85 day incubation periods, duplicate 2g samples of each soil/sludge mixture were extracted with 10 ml of 0.01M $CaCl_2$ on an end-over-end shaker for approximately

24 hours. At the end of the extraction period, each sample was centrifuged. The clarified extract was placed in small plastic bottles and acidified with 2-3 drops of 1N HCl as a preservative. The extracts were stored at room temperature.

At the end of the study period, the cadmium concentrations of the stored samples were determined using a Varian atomic absorption spectrophotometer Model 375. A deuterium lamp was used to determine background correction. Standards of 0.05, 0.1, 0.2, 0.3, 0.5 µg/ml cadmium were used to calibrate the spectrophotometer.

Statistical Analyses

Two-way analysis of variance was performed on the analytical data to determine differences in extractable cadmium across both soils and sludges. Three *a priori* contrasts of extractable cadmium based on the "t" statistic were performed. The *a priori* contrasts were: 1) unlimed acid soils vs. limed acid soils; 2) unlimed acid soils vs. soils with background pH of approximately 6.5; and, 3) limed acid soils vs. soils with background pH of approximately 6.5. All data were analyzed using SAS (Nie. et al., 1975).

In addition, the data in Tables 10.11 and 10.12 were subjected to analysis for least significant difference and Duncan's Multiple Range Test, using a 5 percent confidence level. A regression analysis of cadmium availability as affected by pH was also performed (Nie et al., 1975).

RESULTS

Effects of Sludge and Soils on pH of Sludge/Soil Mixtures

Both the sludge and soil had an effect of the pH of the resulting sludge/soil mixtures. The general effects of each are presented next. The effects of each sludge on Bennington silt loam soil is shown in Figure 10.1.

The addition of sewage sludge increased soil pH in most cases. As expected, the soils that were amended with lime-stabilized Ashland sludge (pH 12.3) tended to have the highest pH at the beginning of the incubation period, while the unamended soils had the lowest pH.

In all but one instance, the addition of sewage sludge to soils resulted in a downward trend in the pH of the soil/sludge mixture during the 85-day incubation period. It should be noted, however, that in many cases, the final pH of the sludge/soil mixtures after the 85-day incubation period had not fallen below the pH of soil prior to the addition of sewage sludge. Thus, the net effect of sewage sludge on soil pH was often an increase in pH. There were also many short-time fluctuations in which the pH of the soil/sludge mixtures increased for short periods (Figures 10.1).

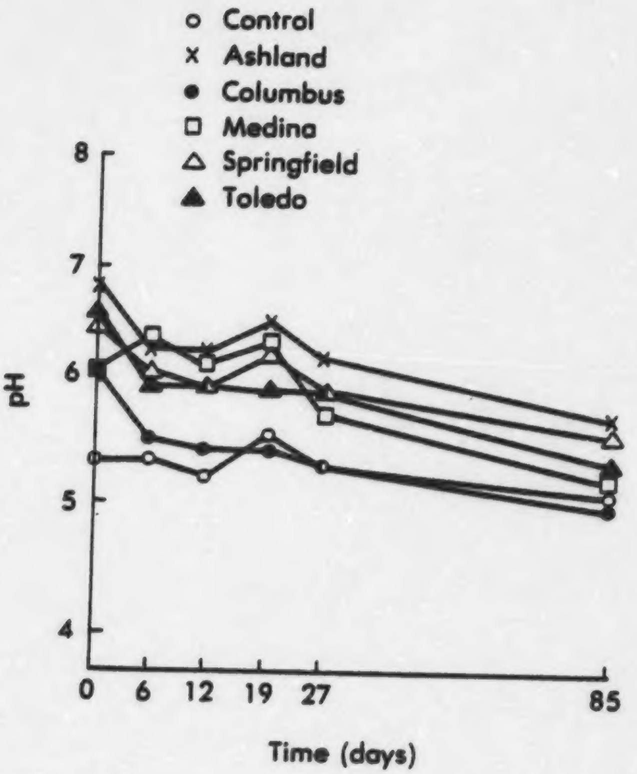

Figure 10.1 Effects of five sewage sludges applied at a rate of 11.2 mt/ha on the pH of a Bennington silt loam soil incubated for 85 days at 24 ± ?C.

The one notable exception to the overall downward trend in pH occurred with the lime-amended Bennington silt loam soil, amended with the lime-stabilized Ashland sludge. In this treatment, the initial pH of the sludge/soil mixture was 7.2. The pH remained at 7.2 after 6 and 12 days incubation. After 19 days incubation, the pH of this mixture had risen sharply to 7.7. It then dropped to pH 6.9 by day 27, and rose to pH 7.3 after an 85-day incubation period. This was the only sludge/soil mixture in which a slight net increase in pH was observed over the 85-day incubation period. It should be mentioned that the soils which were not amended with sludge also experienced a drop in pH over the 85-day incubation period, but, as a rule, the magnitude of this pH change was not as great as those that were amended with sludge (Tables 10.3-10.10).

Magnitude of pH Change

Tables 10.3 through 10.10 show that the magnitude of the pH change of a sludge/soil mixture is strongly influenced by the soil. An example of this effect is demonstrated by the fact that the Toledo sludge/Kokomo soil combination experienced a pH change of only 0.4 (Table 10.4), while the Toledo sludge/Miamian soil combination experienced a pH change of 1.0 units (Table 10.6).

Cadmium Availability

The average pH of all of the treatments of the unlimed acid soils (Bennington and Mahoning) dropped from 6.2 at Day 0 to 5.4 on Day 85. The average extractable cadmium for these same soil/sludge mixtures rose from 0.01 to 0.049 μg/g during the same time period (Figure 10.2). This change in extractable cadmium was found to be significant.

These same two soils (Bennington and Mahoning), when limed to a pH value of approximately 6.5 prior to starting the study, had an average pH drop of only 6.8 at Day 0 to 6.1 at Day 85 (Figure 10.2). The average extractable cadmium for these limed soils was 0.010 μg/g at Day 0 and 0.011 μg/g at Day 85 (Figure 10.2). This change in extractable cadmium was not significant.

The average pH of all of the treatments of the soils with a background pH of approximately 6.5 (Kokomo, Hoytville, Miamian, Spinks) dropped from pH 6.8 at Day 0 to pH 6.2 at Day 85. The average extractable cadmium for all of the soil/sludge mixtures rose from 0.005 μg/g dry weight to 0.019 μg/g dry weight from Day 0 to 85 (Figure 10.2). The change in extractable cadmium was significant at the 5 percent level.

Extractable cadmium for each soil was averaged across all treatments and all incubation periods (Table 10.11). The total extractable cadmium for the unlimed Bennington silt loam soil averaged across all treatments and incubation periods were 0.025 μg/g. The average extractable cadmium for the limed Bennington silt loam soil was

TABLE 10.3. MAGNITUDE OF PH CHANGE OF FIVE SOIL/SLUDGE MIXTURES* INCUBATED FOR 85 DAYS USING A BENNINGTON SILT LOAM SOIL (UNLIMED) AND FIVE SEWAGE SLUDGES

Sludge	pH		ΔpH
	Day 0	Day 85	
Toledo	6.6	5.5	1.1
Columbus	6.1	5.1	1.0
Ashland	6.8	6.0	0.8
Springfield	6.5	5.9	0.6
Medina	6.1	5.5	0.6
Control	5.3	5.1	0.2

* Sludge was applied at a rate equivalent to 11 dry metric tons sludge/hectare.

TABLE 10.4. MAGNITUDE OF PH CHANGE OF FIVE SOIL/SLUDGE MIXTURES* INCUBATED FOR 85 DAYS USING A KOKOMO SILTY CLAY LOAM SOIL AND FIVE SEWAGE SLUDGES

Sludge	pH		ΔpH
	Day 0	Day 85	
Ashland	6.9	6.3	0.6
Medina	6.6	6.1	0.5
Columbus	6.3	5.8	0.5
Toledo	6.5	6.1	0.4
Springfield	6.5	6.3	0.2
Control	6.1	5.9	0.2

* Sludge was applied at a rate equivalent to 11 dry metric tons sludge/hectare.

TABLE 10.5. MAGNITUDE OF PH CHANGE OF FIVE SOIL/SLUDGE MIXTURES* INCUBATED FOR 85 DAYS USING A HOYTVILLE CLAY LOAM SOIL AND FIVE SEWAGE SLUDGES

Sludge	pH		ΔpH
	Day 0	Day 85	
Columbus	6.6	6.1	0.5
Toledo	6.8	6.3	0.5
Ashland	7.2	6.8	0.5
Medina	6.8	6.5	0.3
Springfield	6.9	6.6	0.3
Control	6.5	6.2	0.3

* Sludge was applied at a rate equivalent to 11 dry metric tons sludge/hectare.

TABLE 10.6. MAGNITUDE OF PH CHANGE OF FIVE SOIL/SLUDGE MIXTURES*, INCUBATED FOR 85 DAYS USING A MAHONING SILT LOAM SOIL AND FIVE SEWAGE SLUDGES

Sludge	pH		ΔpH
	Day 0	Day 85	
Ashland	6.4	5.3	1.1
Columbus	6.1	5.0	1.1
Springfield	6.4	5.5	0.9
Control	5.6	4.9	0.7
Medina	6.1	5.6	0.5
Toledo	Not Determined	5.3	--

* Sludge was applied at a rate equivalent to 11 dry metric tons sludge/hectare.

TABLE 10.7. MAGNITUDE OF PH CHANGE OF FIVE SOIL/SLUDGE MIXTURES* INCUBATED FOR 85 DAYS USING A MIAMIAN SILT LOAM SOIL AND FIVE SEWAGE SLUDGES

Sludge	pH Day 0	pH Day 85	ΔpH
Toledo	6.8	5.8	1.0
Columbus	6.6	5.8	0.8
Ashland	6.9	6.3	0.6
Medina	6.8	6.4	0.4
Springfield	6.8	6.5	0.3
Control	6.3	6.0	0.3

* Sludge was applied at a rate equivalent to 11 dry metric tons sludge/hectare.

TABLE 10.8. MAGNITUDE OF PH CHANGE OF FIVE SOIL/SLUDGE MIXTURES* INCUBATED FOR 85 DAYS USING A SPINKS FINE SAND SOIL AND FIVE SEWAGE SLUDGES

Sludge	pH Day 0	pH Day 85	ΔpH
Ashland	7.6	6.4	1.2
Springfield	7.2	6.2	1.0
Medina	7.0	6.2	0.8
Toledo	7.0	6.2	0.8
Columbus	6.6	6.0	0.6
Control	6.5	6.0	0.5

* Sludge was applied at a rate equivalent to 11 dry metric tons sludge/hectare.

TABLE 10.9. MAGNITUDE OF PH CHANGE OF FIVE SOIL/SLUDGE MIXTURES* INCUBATED FOR 85 DAYS USING A BENNINGTON SILT LOAM SOIL (LIMED) AND FIVE SEWAGE SLUDGES

Sludge	pH		ΔpH
	Day 0	Day 85	
Toledo	7.2	6.3	0.9
Columbus	6.7	5.9	0.8
Medina	6.9	6.4	0.5
Springfield	6.9	6.5	0.4
Control	6.5	6.1	0.4
Ashland	7.2	7.3	0.1

* Sludge was applied at a rate equivalent to 11 dry metric tons sludge/hectare.

TABLE 10.10. MAGNITUDE OF PH CHANGE OF FIVE SOIL/SLUDGE MIXTURES* INCUBATED FOR 85 DAYS USING A MAHONING SILT LOAM (LIMED) AND FIVE SEWAGE SLUDGES

Sludge	pH		ΔpH
	Day 0	Day 85	
Columbus	6.6	5.8	0.8
Ashland	7.1	6.4	0.7
Springfield	6.8	6.2	0.6
Medina	6.7	6.1	0.6
Control	6.1	5.9	0.2
Toledo	Not Determined	5.9	--

* Sludge was applied at a rate equivalent to 11 dry metric tons sludge/hectare.

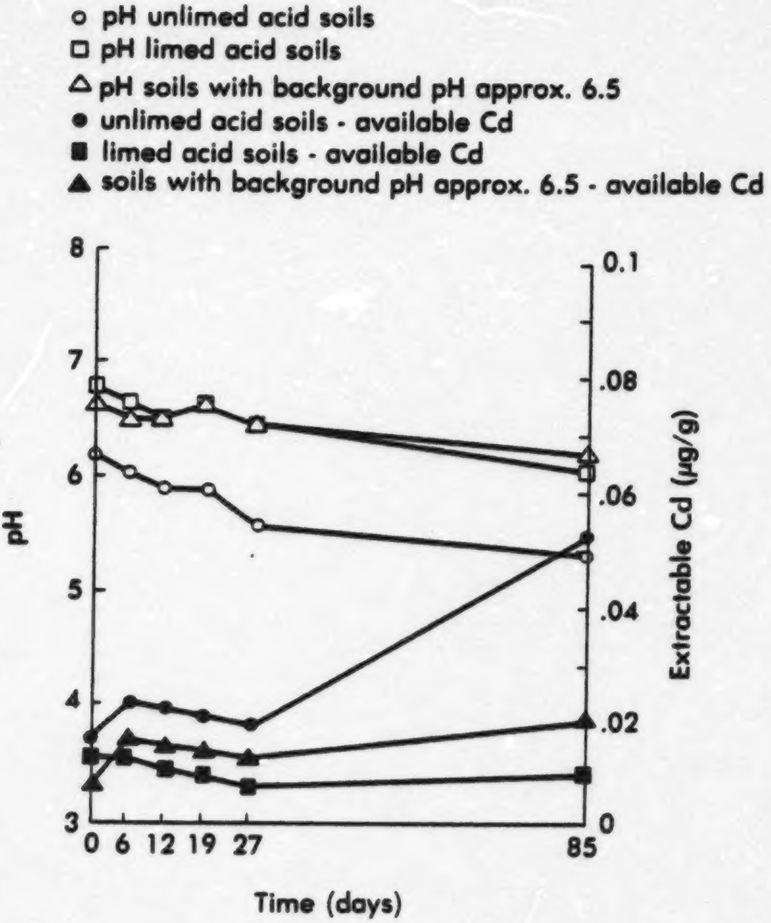

Figure 10.2. Effects of pH and time on 0.01 M $CaCl_2$ extractable Cd of sludge-amended unlimed acid soils, limed acid soils and soils with background pH approximately 6.5.

TABLE 10.11. EFFECT OF VARIOUS SLUDGE/SOIL MIXTURES* ON AVERAGE EXTRACTABLE CADMIUM DURING AN 85 DAY INCUBATION PERIOD

Soil	Sludges						
	Control	Ashland	Columbus	Medina	Springfield	Toledo	Average Cd
				µg/g			
Bennington	0.014	0.015	0.061	0.024	0.012	0.024	0.025
Kokomo	0.015	0.009	0.024	0.014	0.004	0.009	0.013
Hoytville	0.007	0.024	0.024	0.012	0.022	0.019	0.018
Mahoning	0.019	0.022	0.037	0.015	0.022	0.032	0.025
Miamian	0.007	0.01	0.014	0.02	0.014	0.009	0.012
Spinks	0.009	0.01	0.02	0.003	0.010	0.009	0.010
Bennington and lime	0.003	0.009	0.032	0.003	0.000	0.01	0.010
Mahoning and lime	0.000	0.015	0.022	0.02	0.000	0.014	0.012
Average	0.009	0.014	0.029	0.014	0.011	0.016	

* Sludge was applied at a rate equivalent to 11 dry metric tons sludge/hectare.

0.010 μg/g. The average extractable cadmium for the unlimed Mahoning silt loam soil was 0.025 μg/g; while the average extractable cadmium for the limed Mahoning silt loam was 0.012 μg/g.

Thus, liming these soils did lower the average extractable cadmium concentration in each soil. This change in extractable cadmium concentration between the limed and unlimed soils was significant at the 5 percent level.

When the data presented in Table 10.11 was subjected to a two-way analysis of variance, it was found that there were significant soil and sludge effects on cadmium availability. However, there were no statistically significant interaction effects.

The average extractable cadmium across all soils and sludges (Table 10.11) was subjected to an analysis of least significant difference and a Duncan's Multiple Range Test using a 5 percent confidence level. The cadmium availability of the unlimed Bennington and Mahoning soils was found to be significantly different from all other soils except Hoytville. There were no significant differences between the Kokomo, Miamian, Spinks, Bennington + lime, Mahoning + lime or Hoytville soils.

No significant differences in extractable cadmium were found for the soils averaged across Ashland, Medina, Springfield, Toledo sludges and the control (unamended) soil. The extractable cadmium in the Columbus sludge/soil mixtures was significantly different from all other sludges (Table 10.11). For the unlimed acid soils, there was no significant difference in the amount of extractable cadmium for Day 0, 6, and 27 (Table 10.12). However, the extractable cadmium for Day 85 was significantly different from all of the other days.

For the soils with a background pH of approximately 6.5, Day 0 was significantly different from all other days. There were no significant differences in extractable cadmium between any incubation periods of the limed acid soils.

Table 10.13 and Figure 10.3 points out that there was no direct linear relationship between the amount of cadmium added to soils in sewage sludges, and the amount of cadmium that became available for plant growth, after an 85-day incubation period.

DISCUSSION

This study points out that the effects of soil pH on sludge cadmium availability are not fully understood, particularly where acidic soils are involved. In general, all of the sludges tended to initially raise the pH of the soil. This was not surprising considering that the pH of the sludges varied from pH 6.7 in the Medina sludge to pH 12.3 in the Ashland sludge. With very few exceptions, the sludges were more alkaline than the soils so they tended to raise the initial pH.

TABLE 10.12. EFFECT OF INCUBATION TIME ON CADMIUM AVAILABILITY FOR SEWAGE SLUDGE AMENDED*, LIMED ACID SOILS, UNLIMED ACID SOILS, AND SOILS WITH A BACKGROUND PH OF APPROXIMATELY 6.5

Soil	Extractable Cd (µg/g)			
	Day 0	Day 6	Day 27	Day 85
(Limed) Acid Soils	0.010	0.011	0.006	0.011
(Unlimed) Acid Soils	0.010	0.022	0.018	0.049
Soils With Background pH of Approx. 6.5	0.005	0.016	0.012	0.019

* Sludge was applied at a rate equivalent to 11 dry metric tons sludge/hectare.

TABLE 10.13. RELATIONSHIP BETWEEN AVERAGE CADMIUM ADDED TO EIGHT SOILS IN FIVE DIFFERENT SEWAGE SLUDGES AND EXTRACTABLE CADMIUM AFTER AN 85-DAY INCUBATION PERIOD AT ROOM TEMPERATURE (APPROX. 25C)

Sludge	Added Cd (µg Cd/g)	Extractable Cd Minus Background Cd* (µg Cd/g)	Percent Added Cd Recovered as Extractable Cd
Ashland	123	0.005	0.004
Columbus	285	0.020	0.007
Medina	18	0.005	0.028
Springfield	190	0.002	0.001
Toledo	59	0.007	0.012

* Background Cd concentration was 0.009 µg Cd/g soil.

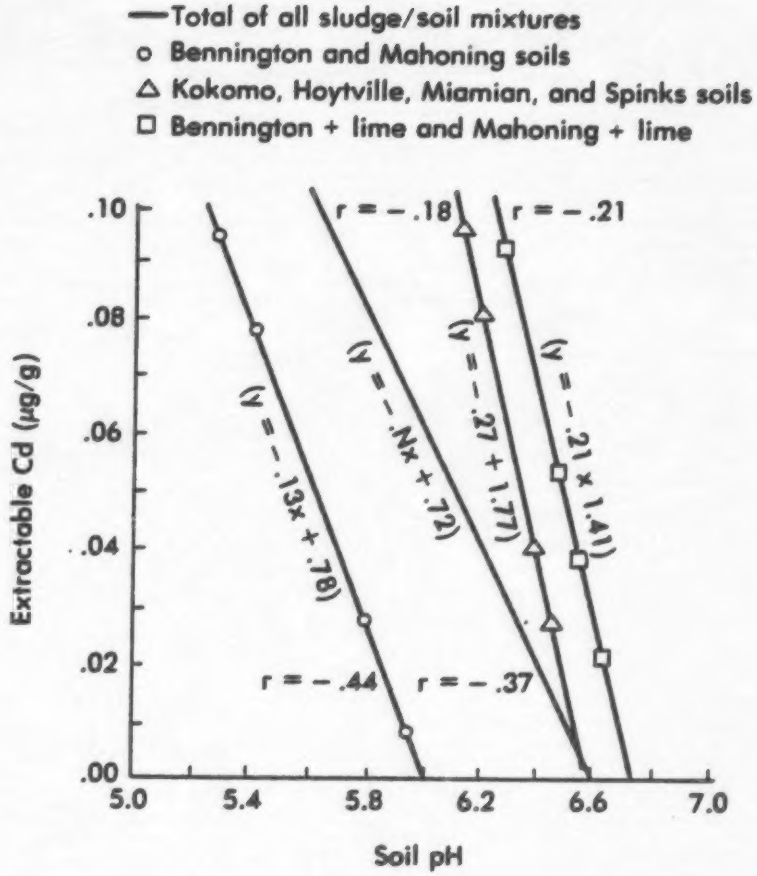

Figure 10.3. Linear regression of 0.01M $CaCl_2$ extractable Cd versus soil pH for all combinations of 8 different sludges and 5 different soils.

Results of this study show that, under the prescribed conditions, the availability of sludge-borne cadmium increased as pH decreased. However, the average cadmium availability did not increase significantly until the pH of the sludge/soil mixture dropped below approximately 6.0.

The only group of soils that experienced an average pH drop to under 6.0 were the unlimed acid soils (Bennington and Mahoning). There was also a greater increase in extractable cadmium in these soils than in either the soils with a background pH approximately 6.5 or the limed acid soils.

From this study, it appears that the only direct influence of increased incubation time is a lowered pH which in turn affects cadmium availability. If time in itself had a significant effect, one would expect that, as incubation time increased, cadmium availability would increase regardless of the soil/sludge mixtures used. This is not the case (Table 10.12 and Figure 10.2).

Over the long run (85 days), the pH of the soil/sludge mixture decreased. There exists an unproven possibility that the pH could continue to decrease with additional time, and eventually increase cadmium availability in the other two soil groups.

As mentioned earlier, the moisture content of the sludge/soil mixtures was not controlled. The moisture content was above 1/3 bar (field moisture content) for those sludges that have a low percent solids content, such as the Medina sludge. In addition, the sludge/soil mixtures tended to partially dry out during the course of the experiment. No radical moisture effects were observed during the study. Since the same sludges were used on all of the soils and differences were found in the cadmium availability of the mixtures, it can be stated that soil moisture content was not a major factor. If there were some slight effects, they were overshadowed by the pH effect.

An additional complication to the evaluation of the effects of soil pH on sludge-borne cadmium is the fact that each sewage sludge has unique chemical, physical, and microbiological characteristics. Therefore, the reactions that take place when a sludge is added to a soil are dependent on the characteristics of both the soil and the sludge. There are reports in the literature (CAST, 1980) which indicate that the effect of the chemical form of the cadmium in the sludge may have significant effects on cadmium availability. Other sludge-related factors, such as degree of decomposition, sludge pH, percent solids, other elements present, etc., also have a confounding effect on any study which is designed to determine the reasons for variable cadmium availability.

The results of this study show that there may be some merit in the EPA requirement that the pH of the sludge/soil mixture be controlled. However, the requirement that the pH of the sludge/soil mixture be raised to 6.5 may be too stringent. The study revealed trends that show that cadmium did become more available as pH decreased in the unlimed acid soils, and very slightly more available in the soils with a background pH approximately 6.5, while the cadmium did not become more available in the limed acid soils. Extractable cadmium increased most sharply only after the pH of the sludge/soil mixture had fallen to some point under 6.0.

The Ohio State University "Agronomy Guide" (1980-81) points out that some highly buffered soils may require over 6.7 mt/ha in order to raise soil pH from 6.0 to 6.5. The cost of lime is now often as high as $20 per metric ton; thus, it could cost 134/ha to bring a highly buffered soil from pH 6.0 to 6.5 to meet EPA requirements.

In recent years, there has been an effort to persuade municipalities to apply sewage sludge at very low rates (< 11 dry metric tons sludge/hectare) and thus reduce the potential risk of runoff or groundwater contamination. Many municipalities have been willing to use the low rates because there has been an ample number of private landowners near the treatment plants who were willing to accept sewage sludge. There has also often been no required site preparations before sludge has been applied. Therefore, the municipality had no money invested in an application site, and as long as other sites were available in the area, there was no incentive to overload any site with a high sludge application rate. If municipalities are forced to amend soil pH prior to applying sewage sludge, they will be forced to invest money for lime in each disposal site that they use. Cost of sludge disposal will be strongly influenced by the amount of land that is limed for disposal. Consequently, there will be an economic incentive for municipalities to overload those sites that they have limed and reduce the number of hectares that need to be limed each year.

SUMMARY

The results of this study demonstrated that:

- Initially, the addition of sewage sludge to soils tended to raise soil pH.
- Over an 85-day incubation period, the pH of the sludge/soil mixtures decreased. However, the pH of the mixtures did not always drop below the pH of the unamended soils alone.
- Extractable cadmium of the limed acid soils was not increased as pH of the sludge/soil mixtures decreased, over an 85-day incubation period.
- Extractable cadmium of the soils with a background pH of approximately 6.5 experienced a very minor increase in cadmium availability as the pH of the soil/sludge mixtures decreased over an 85-day incubation period.
- Extractable cadmium of the unlimed acid soils (background pH under 6.0) was significantly increased as pH of the sludge/soil mixtures decreased over the 85-day incubation period. The most dramatic increase in cadmium availability in these sludge/soil mixtures occurred after the pH of the mixtures had dropped to somewhere under 6.0.

- Statistical analysis of the data showed that cadmium availability of the sludge/soil mixtures was significantly affected by the sludge and the soil. However, there were no significant interaction effects.

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SECTION 11

HEALTH EFFECTS OF MUNICIPAL SEWAGE SLUDGE APPLICATION ON OHIO FARMS

C. Richard Dorn, D.V.M., M.P.H.*
Chada S. Reddy, B.V.Sc., M.S., Ph.D.*
David N. Lamphere, D.V.M., Ph.D.*
John V. Gaeuman, M.D.#
Richard Lanese, Ph.D.#
*Department of Veterinary Preventive Medicine
#Department of Preventive Medicine
The Ohio State University
Columbus, Ohio 43210

SUMMARY

A 3 year prospective epidemiologic study was conducted on 47 farms receiving annual sludge applications and 46 control farms in 3 geographic areas of Ohio. All the sludge was from municipal sewage treatment facilities and had been treated either anaerobically (Franklin-Pickaway Counties and Clark County) or aerobically (Medina County). The sludge was spread by applicator equipment at the rate of 2-10 dry metric tons per hectare. One hundred sixty four persons (78 families) on sludge farms and 130 persons (53 families) on control farms participated by cooperating with monthly questionnaires concerning their health and their animals' health, annual tuberculin testing, and quarterly blood and fecal sampling for microbiological testing. The risks of respiratory illness, digestive illness, or general symptoms were not significantly different between sludge farm and control farm residents. Similarly, there were no observed differences in disease occurrence among domestic animals on sludge and control farms. There were no persons who converted from tine test negative to tine test positive after sludge had been applied to their farm. The frequency of serological conversions of a 4 fold or greater rise in antibody to a series of viral antigens and the frequency of associated illnesses were similar among persons on sludge and control farms. The absence of observed human or animal health effects resulting from sludge application in this study of Ohio farms was based upon low sludge application rates which were in accordance with Ohio and USEPA guidelines. Caution should be exercised in using these data to predict health risks associated with sludges containing high levels of hazardous disease agents and with higher sludge application rates and larger acreages treated per farm than used in this study.

INTRODUCTION

The amount of sewage sludge for disposal has increased greatly since the U.S. Government required that all publicly owned sewage treatment plants include secondary treatment. Furthermore, the Water Pollution Control Act Amendments of 1972 required that an assessment of alternative treatment technologies, including sludge recycling to the land, be considered before construction grants could be awarded. The Marine Protection, Research and Sanctuaries Act of 1972 curtailed the routine practice of dumping sludge into the ocean (Galen, 1980). The alternative of incinerating sludge requires large amounts of energy and it must be done in compliance with the Air Quality Act of 1967. Therefore land application and landfills have been used more in recent years. Suitable landfill sites are, however, being exhausted. Thus sludge application on farmland has advantages for the municipalities, in addition to the economic advantages to the farm owner and operator of using the sludge as a fertilizer and as a soil conditioner.

Municipal sewage sludge has been applied to farmland for many years; however, there remain questions about the human health and animal health consequences of this practice. Potentially harmful microbiological agents and chemical substances in sewage sludge have been described in several reviews (Burge and Marsh, 1978; Epstein and Chaney, 1978; Pahren _et_ _al_., 1979; Jones, 1980; Kowal and Pahren, 1980; WHO, 1981). Sewage treatment employees who were occupationally exposed to sludge, in addition to raw sewage, were the subject of several investigations; however, many of these studies can not be adequately evaluated due to lack of controls (Clark _et_ _al_., 1976). A study of Paris sewage plant workers found that they had a higher risk of amaebiasis and giardiasis as compared to the general population (Doby _et_ _al_., 1980). Hepatitis A infection in Copenhagen workers has also been correlated to sewage exposure (Shrink _et_ _al_., 1981). The health of sewer workers, sewage treatment workers and their families was monitored in several U.S. cities and no evident adverse health effects of occupational exposure were observed (Clark _et_ _al_., 1980). A seroepidemiologic study of sewage treatment workers failed to show any evidence of increased risk of infection as measured by an increase in antibody titers (Clark _et_ _al_., 1978).

Epidemiologic studies of persons living near sewage treatment facilities have also been conducted. In a study of acute illness among population groups at varying distances from the Tecumseh Wastewater Treatment Plant in Michgan, the higher illness rates were related more to socioeconomic status of the families than to proximity to the wastewater treatment plant (Fannin _et_ _al_., 1980). A study of persons living near a new activated sludge plant in Illinois found a higher incidence of skin disease and severe gastrointestinal symptoms after the plant became operational (Johnson _et_ _al_., 1980). However, antibody tests for 31 human enteric viral antigens and attempted isolations of many pathogenic bacteria, parasites and viruses yielded virtually no clinical evidence of infectious disease effects associated with the sewage treatment aerosol. In Oregon, school attendance records of children living near a wastewater treatment facility were studied (Camann _et_ _al_., 1980). The sewage treatment aerosol had no adverse effect on communicable disease incidence as discerned from total school absenteeism. The health effects of aerosols emitted from an activated sludge plant in Skokie, Illinois were studied over an 8 month period (Northrup et al., 1980). No correlations were reported between the exposure indices and rate of self-reported illnesses or of bacterial or viral infection rates determined by laboratory analysis. Of the 246 families studied only a few were exposed to the highest pollution levels.

Several epidemiologic reports provide accounts of enteric infections resulting from the use of untreated wastewater in the cultivation of crops that are subsequently eaten raw (Geldreich and Bordner, 1971; Hoadley and Goyal, 1976; Sepp, 1971). A large retrospective study of kibbutzim in Israel exposed to slow-rate land treatment with nondisinfected oxidation pond effluent was reported in 1976 (Katzenelson et al.) and in 1980 (Shuval and Fattal). The 1983 final report of this study did not confirm the 1980 findings of increased incidence of typhoid fever, salmonellosis, shigellosis and infectious hepatitis in the exposed kibbutzim, although a small significant excess risk of total enteric disease was found during the effluent irrigation period (Shuval et al., 1983). There have been no previously reported studies of the human health effect of land application of sewage sludge.

The health of livestock on farms where sludge is applied is also a concern. The major pathogenic agent of concern is Salmonella acquired by animals grazing on sludge applied land. Calves grazing pastures to which slurry containing 10^6 Salmonella dublin organisms/ml had been applied became infected (Taylor and Burrows, 1971); however, a later study using 10^5 S. dublin/ml failed to infect calves when allowed to graze 7 days after spreading (Taylor, 1973). Goats raised on corn silage grown on sludge-amended land did not become infected with Salmonella even though Salmonella was present in the sludge (Ayanwale et al., 1980). Studies of carrier rates and serotypes of Salmonella in cattle grazing on sludge treated pastures in Switzerland has indicated a positive association and a cycle of infection from man to sludge to animals to man (WHO, 1981). Similar evidence was reported from the Netherlands, but not from the United Kingdom where compulsory reporting of salmonellosis is required.

Cattle serve as the intermediate host for Taenia saginata of man. The Taenia ova can survive from several days to 7 months (Babayeva, 1966). Taenia saginata cysticercosis in cattle due to ingestion of T. saginata ova has been associated with exposure to human sewage in Australia (Rickard and Adolph, 1977), in the United Kingdom (Macpherson et al., 1978) and to sewage sludge in Virginia (Hammerberg et al., 1978). Thirty seven cysticercosis cases were found among calves exposed to raw sewage flooding pastures on a farm on which sewage sludge had also been applied (Fertig, 1982). Seven cysticercosis cases occurred 2 years later on the same farm when only sewage sludge was the likely source of infection (Dorn, 1983).

Livestock are also exposed to chemicals in sludge by direct ingestion of sludge adherent to vegetation or by ingestion of feeds grown on agricultural lands where sludge was applied. Animal food products consequently serve as an avenue for the translocation of chemical compounds, such as heavy metals, to human beings. One study reported significantly higher ($P < 0.05$) levels of cadmium in the liver and kidneys of cows exposed to a publicly owned sludge recycling site as compared with controls (Kienholz et al., 1977). Fitzgerald (1980) reported that the average kidney cadmium level in 39 cows grazing on sludge treated pastures was 40.7 µg/g. Several studies have examined tissue residue levels in cattle (Kienholz et al., 1979; Bertrand et al., 1980; Boyer et al., 1981), swine (Hammell et al., 1977; Hansen and Hinesly, 1979; Beaudouin et al., 1980) and sheep (Smith et al., 1977) fed sewage sludge or sludge fertilized feed as part of their ration.

Mycobacterium is another pathogen which is isolated from sewage sludge (Jones et al., 1981). Mammalian as well as atypical Mycobacterium strains in sludge could infect or sensitize exposed human beings and livestock species. Even though sensitized individuals may not become ill, they would react to the tuberculin test resulting in diagnostic and possibly economic problems for livestock owners.

Since no systematic investigation of human and livestock health effects of sewage sludge application on privately owned farms has been conducted, the study described in this report was initiated. The study was a cooperative undertaking between the U.S. Environmental Protection Agency (EPA), the Ohio Farm Bureau Federation, the Ohio Department of Health, and the Ohio State University. The objectives of the research described in this Section were to: (1) monitor the health status of human beings and animals living on sludge receiving and control farms, (2) determine if sludge application is associated with higher illness rates, and (3) determine if sludge application on farms is associated with conversion of tuberculin reactions from negative to significant.

METHODS

Selection of Participating Farms:

Three locations in various areas of Ohio were selected for the health study. These locations were selected because they represented different geographic areas of the state; treatment facilities officials were interested in disposing of their sludge via farmland application; and each produced enough sludge to accomodate large numbers of participating farms. The three locations were Medina County, Columbus area (Franklin and Pickaway Counties), and Clark County (Figure 11-1). More detailed information about each of these sites is presented in Section 1.

The Ohio State University Cooperative Extension staff in each involved county compiled a listing of all farms over 20 acres (8.1 ha) in size that might participate in the project. The recruitment of farms began in the Spring of 1978. The farm owners and operators were contacted and invited to attend an educational meeting conducted by the project staff. At that meeting, those persons interested in having their farms considered for sludge application completed a detailed questionnaire about their farming operation and livestock. From these respondents, a list of eligible farms was developed after eliminating certain farms because of their distance from the sewage treatment facility, proximity to a stream, or other environmental considerations. All dairy farms were excluded in accordance with an Ohio

Department of Health recommendation based on their interpretation of regulations which prohibit human fecal waste from being applied on farms producing Grade A milk.

For each location, the farms in the eligible pool were assigned, using a table of random numbers, to either receive sludge or serve as a control farm. Because of the process of sludge hauling and activation of participating farms, 2 to 10 farms in a group were assigned at a time to either receive sludge or to serve as a control. Thus for each sludge farm, except one in Medina County, there was a control farm from the same county further matched in the sense that their pre-sludge and post-sludge periods of observation corresponded (Figures 11-2, 11-3). Sludge applications were repeated approximately once a year. Since the farms which were chosen to receive sludge benefitted economically from the fertilizer and soil conditioning properties of the sludge, the control farms received incentive payments of $150, one at the beginning and the other at the end of the study.

The farms designated to receive sludge were visited by an agronomy team which conducted an agronomic survey of the farm, collected soil samples, reviewed present and planned crop rotations, and made recommendations concerning time, location, and amount of sludge application. The site of sludge application was inspected by Ohio EPA and approved to receive sludge prior to sludge application. The sludges used in Franklin-Pickaway Counties and Clark County were anaerobically treated and in Medina county the sludge was aerobically treated. The sludge was spread by applicator equipment at the rate of 2-10 dry metric tons per hectare. Only selected fields or parts of fields received sludge. The average acreage of the farms was 47 for Franklin-Pickaway and 15 for Medina and Clark.

The farm owners and operators were visited prior to sludge application by a health team consisting of an epidemiologist and a nurse. The health studies were then explained in greater detail, informed consent forms were signed, an initial baseline health questionnaire was completed for each farm resident. The questionnaire consisted of the following:

1. Farm information form: contained information on the location of the farm, listing of people living and/or working on the farm on a regular basis, and farm environment.

2. Human exposure form: contained individual participant's work schedule both on and off the farm, nature and source of food, contact with animals and previous medical history including illnesses and vaccinations. Additional information on immunization history, smoking history, and chronic illnesses was collected on an annual basis.

3. Human illness form: recorded symptomology and course of a participant's illness.

4. Animal exposure form: described the environment, source of food, time spent on the sludge field and illnesses for each livestock unit.

5. Animal illness form: showed the nature and duration of illness in each livestock unit.

Followup questionnaires on animal and human health were completed by telephone at approximately one month intervals, beginning with the initial interview.

Tuberculin Testing:

Each participant was given a tuberculin tine test (Tuberculin, Old, Tine Test, Lederle Laboratories, Pearl River, N.Y.) by the project nurse. The test was read by the test subject at 72 hr post-infection. The mail-back postcard provided with the tuberculin tine test kit was used for recording and reporting the results.

A tine test significant reaction was defined as any induration of 5mm or more. Prior to September 1979 those reporting a reaction to the tine test were referred to their family physician for further evaluation. After September 1979 individuals who reported a significant reaction to the tine test were given a Mantoux test using human strain Purified Protein Derivative (PPD) produced by Connaught Laboratories Ltd., Willowdale, Ontario, Canada. In subsequent testing only the Mantoux test was given to these individuals. The Mantoux test significant reaction was defined as an induration of 10 mm or more. Those positive to the Mantoux test were examined by thoracic radiography for confirmation. The tuberculin testing was performed on all participants at yearly intervals to evaluate possible conversions from negative to positive (significant). Sludge application on these farms was coordinated so that the first (baseline) tuberculin testing was done within one week before the date of first sludge application.

Microbiology Sampling:

Samples of blood and feces were collected, when the first tine test was administered, to obtain baseline microbiological information. Thereafter, samples were obtained at 4 month intervals. The procedures for transporting, processing, and identifying the paired baseline and followup serum samples for the first and second sludge applications are described in Section 15. For associating illnesses with specific seroconversions a series of 21 viral antigens were used. Only those seroconversions that occurred within a period of 6 months after sludge application were considered to be possibly due to sludge exposure. A seroconversion was a 4-fold increase in antibody titer to a specific viral antigen between 2 consecutive serum samples.

Data Analysis:

Data from human and animal questionnaire responses were coded and entered into an Amdahl 470 computer for future retrieval and analysis. The questionnaire data and comparable demographic data obtained from the 1970 Census of Agriculture were displayed descriptively. Because human beings, animal units and the numbers of animals existed on the farms varying lengths of time, person-years at risk, unit-years at risk and animal-years at risk were computed. Person-years at risk values were calculated by adding participation periods for all individuals at risk. The period of observation started on the date of the first interview following sludge application and ended at the termination of the study on that farm. This descriptive examination of the data used the longest possible observation period in order to include illnesses due both to infectious agents and chemical agents which accumulate with additional sludge applications and remain in the environment for long periods of time. Subsequent data analyses focused on the infectious disease transmission hypothesis and were limited to the period immediately following sludge applications, e.g. 7 weeks or up to 3 months.

For controls, participation started on the date of interview following the date of sludge application on its corresponding sludge farm. The number of unit-years at risk was calculated by adding the number of weeks of data contribution by each unit for each interview and dividing by 52. The number of animal-years at risk was calculated by adding the total number of weeks of data contribution by all animals within each unit at the time of each interview and dividing by 52. An animal unit was defined as a group of animals of the same species and type of operation under the management of a single family. These values were then used as denominators in calculations of illness rates. The following equations were used:

$$\text{Human illness rate per 100 person-years at risk} = \frac{\text{No. of new illnesses}}{\text{No. of person-years at risk}} \text{ X } 100$$

$$\text{Animal illness rate per 100 unit-years at risk} = \frac{\text{No. of units affected}}{\text{No. of unit-years at risk}} \text{ X } 100$$

$$\text{Animal illness rate per 100 animal-years at risk} = \frac{\text{No. of animals ill}}{\text{No. of animal-years at risk}} \text{ X } 100$$

Two panels of persons having no knowledge of the actual data being collected were asked to group the illness symptoms and signs measured in the human and the animal questionnaires, respectively, into clinical entities suitable for data analysis. The panel for the human data consisted of 2 infectious disease specialists, a toxicologist and an epidemiologist. The human symptoms were combined into the following groups: (1.) General: fever, headache or generalized muscular aches and pains, (2.) Digestive: nausea or diarrhea, (3.) Upper respiratory: runny nose, sore throat, nasal congestion or hoarseness, and (4.) Lower respiratory: chest congestion or cough. The panel for the animal illness data consisted of a veterinary clinician and an epidemiologist. Selected animal signs were combined into the following groups: (1.) Digestive: constipation, diarrhea, or blood in feces and (2.) Respiratory: cough, nasal discharge, or difficult breathing.

The human illness data were subjected to statistical analysis using the multiple logistic regression method (Breslow and Day, 1980; Kleinbaum, 1980) using both the individual and the above combined symptom categories. The multiple logistic regression model has been one of the approaches to investigate the effects of independent variables on the risk of developing disease symptoms. Most of the time, in these studies, the dependent variable is binary in nature (d=1 for the presence of the response variable (illness or symptom) and d=0 for the absence of the response variable). The independent variables can be defined as $x_1, x_2, \ldots x_R$, then

$$\Pr(d = 1 | x_1, x_2, \ldots, x_R) = \frac{1}{1 + \exp\left[-\alpha - \sum_{j=1}^{R} \beta_j x_j\right]}$$

β represents the regression parameters and α is the intercept. Pairwise matching was used in this study to investigate the effects of certain independent variables on the risk of developing a symptom. The sludge farms were matched with control farms on the basis of entry-date into the study. There were 46 matches for this analysis; one farm in Medina County was eliminated because there was no match.

The following table shows the notational scheme of the matched pair study in which R risk variables are under evaluation. The x's refer to the risk variables for the cases, while the y's are those for controls. The first subscript indexes the pair while the second subscript denotes the risk variable.

Notation for matched pair case-control study, where the n_d discordant pairs preceed the $n-n_d$ concordant pairs.

Pair	Case	Control
1	$(x_{11}, \ldots x_{1R})$	$(y_{11}, \ldots y_{1R})$
2	$(x_{21}, \ldots$ x2R	$(y_{21}, \ldots y_{2R})$
.	.	.
.	.	.
.	.	.
n	$(x_{n1}, \ldots x_{nR})$	$(y_{n1}, \ldots y_{nR})$

For matched pair case-control data, the conditional likelihood is determined by computing the product of n_d conditional probabilities i.e. one conditional probability for each discordant pair. The product of these n_d probabilities, is the conditional likelihood. Since in this study only one control farm is matched to each sludge farm, the conditional likelihood can be written as follows:

$$\prod_{i=1}^{n_d}\left[\frac{1}{1 + \exp\left(\sum_{j=1}^{R} \beta_j (y_{ij} - x_{ij})\right)}\right]$$

Holford et al. (1978) treat this as a conventional likelihood function applying standard likelihood results to make inferences on $\beta_1, \ldots \beta_R$. The coefficients can easily be interpreted in terms of odds ratios, which are estimates of the relative risk. In the present study the exponentiated β is the odds of reporting a symptom with each unit change of the independent variable.

The human data were analyzed separately for the first and the second sludge applications. This analysis used farm as the unit of observation rather than individuals to avoid bias due to clustering of illnesses within farm families. The period of observation following sludge application was 3 months or approximately 3 interviews corresponding to the time period when infectious diseases due to sludge exposure might be expected. The following variables were accounted for in the multiple logistic analysis: sludge or control farm, number of weeks of observation, and number of persons in each age group (0-4, 5-12, 13-21, 22-59 and $\geq$ 60. The response variable was one or more reported illnesses in the farm family during the period of observation.

The statistical analysis of the animal illness data was limited by small numbers of some types of animal units and the absence of livestock, pets or both on some farms. Therefore it was not possible to perform a matched-pair analysis. Using the animal unit as the basis of measurement, the X^2 test was used to screen the data for possible associations between illness signs (single or combined) and use of sludge on the farm. For this calculation, illnesses experienced during the first 3 months after the first sludge application and after the second sludge application were analyzed separately. A program for testing of followup data with animal unit-time denominators was also applied to illness rates per 100 unit-years at risk to determine if any rates were significantly different on sludge farms compared to those on control farms (Rothman and Boice, 1979).

Comparisons were also made between sludge farms and control farms to explore other independent variables which might have resulted in significant confounding effects on human and animal illness. For these comparisons the X^2 test was used for categorical variables and the F test (analysis of variance) was used for continuous variables. The 5% level of significance was used for all statistical tests.

RESULTS AND DISCUSSION

For the combined 3 locations, a total of 47 farms received sludge and 46 farms served as controls (Table 11-1). As more than one family was often associated with a single study farm, the sludge farms had 78 families with 200 eligible persons and the control farms had 53 families with 174 eligible persons. A person eligible to participate was one who resided on or regularly visited a designated project farm. For the sludge farm group, eligible persons had to reside on or visit regularly the site where the sludge was applied.

Of the eligible persons, 164 persons on sludge farms and 130 persons on control farms initially participated (Table 11-1). Thirty six individuals that refused to participate were from sludge-receiving farms; 18 were males and 18 were females. Forty four individuals that refused to participate were from control farms; 26 were male and 18 were female. Factors including delays in sludge applications and withdrawal from participation due to personal reasons changed the duration of participation among farms. Considering the date of first sludge application as the beginning of the project (the same date for the time-matched control), all farms and participants completed at least one year of participation. Thirty six sludge farms completed 2 years and 13 completed 3 years. Thirty seven control farms completed 2 years and 13 completed 3 years. The small number of participating farms and persons completing 2 and 3 years is due primarily to late start up times and voluntary withdrawal from the study (Figures 11-2 - 11-3). The farms in Medina County started first, followed by Franklin-Pickaway Counties' farms and then by Clark County farms. The largest numbers of participants were in Franklin-Pickaway Counties; both Medina County and Clark County had approximately the same number of participants (Tables 11-2 - 11-4). None of the Clark County farms participated the third year due to late recruitment.

HUMAN POPULATION CHARACTERISTICS

Sex

There were more males than females in both the sludge and control groups, 1.37:1 and 1.28:1 respectively (Table 11-5). This pattern was found for Franklin-Pickaway Counties and Clark County but not for Medina County which had approximately the same numbers of males and females (Tables 11-6 - 11-8). The general population sex ratio in each of the 3 localities and in the composite population slightly favored the males (Tables 11-9 - 11-12).

Age

Most of the people on both sludge and control farms were in the 50-59 year age groups (Table 11-5). In the 0-19 year age interval there was proportionally fewer persons on both sludge farms and control farms than enumerated in the 1970 census (Table 11-9). Conversely, there was proportionally more persons in the 50-69 year age group than enumerated in the 1970 census. This pattern was consistent for all three locations (Tables 11-6 - 11-8; 11-9 - 11-12).

Number of persons on farms and in families

As shown in Table 11-1, there were 47 sludge receiving farms and 46 control farms; however there were 78 families in the sludge group as compared with 53 in the control group. It therefore becomes important to consider the distribution of persons in each farm and in each family unit. There were far more single person families in the sludge group as compared to the control group, 31 *vs.* 12 respectively (Table 11-13). This disparity was contributed to by Franklin-Pickaway Counties and Clark County but not Medina County (Tables 11-14 - 11-16).

In Franklin-Pickaway Counties this abundance of single person family units was due in part to a plant nursery farm which regularly employed 7 male laborers. Three of these persons, but not their family members, were exposed to the sludge. Therefore they were considered to be single person family units for the purpose of the study. In Clark county, one farm had three families with only one adult male participating in each family. The frequencies of families with 2,3, etc. persons were similar for sludge and control groups in the 3 study locations (Table 11-13).

Race

All of the participants were white Americans.

Smoking Status

Because of the association of smoking with several different disease conditions and because of its effect on cadmium intake, the smoking history of each participant was determined. A smoker was defined, for the purposes of this study, as someone who had smoked cigarettes at any time before the final interview. Smokers who quit smoking or participants who started smoking during the post-sludge project period were all considered smokers. A non-smoker was someone who had never smoked cigarettes before the final interview. These definitions were used because they would represent extremes and thus increase the likelihood of observing differences between smokers and non smokers if they existed. Where the subcategory size was larger than one or 2, there was very close agreement between sludge and control groups in the proportions of smokers and non smokers in male and female groups and in various counties (Table 11-17). There were higher proportions of smokers among men than among women.

Immunization History

Polio --

One person on a sludge farm in Medina County had poliomyelitis (polio) prior to the start of the study on their farm (Table 11-18). No other sludge farm or control farm participant had a history of polio. Approximately 90% of both combined sludge and control groups had been immunized. Clark County had the highest level of polio vaccination followed by Franklin-Pickaway Counties and Medina County (Tables 11-19 - 11-21). No cases of polio were reported during the study.

Measles --

Approximately one-fourth of the combined sludge and control groups had been immunized for common measles (rubeola). The measles immunization level was higher for Franklin-Pickaway Counties than for the other counties (Tables 11-18 - 11-21). No cases of measles were reported during the study.

Rubella --

Among the combined sludge group and the combined control group, 58.3% and 59.2%, respectively, had previously had rubella (Table 11-18). The immunization level for rubella was approximately the same as for measles. No cases of rubella were reported during the study.

Distribution of Time Spent On-farm and Off-Farm

Each participant was asked about the amount of time they spent in field work, in livestock work and in off-farm activities during each monthly interview. Their monthly responses were averaged for each three month season over the three year period. On the sludge farms the amount of the field work that was on sludge applied land was also determined. Approximately 13% of their field work was performed on sludge applied land over all seasons (Table 11-22). Proportionally less time was spent on sludge land in Clark County than in the other counties (Tables 11-23 - 11-25).

Most of the field work was performed in Spring, Summer and Autumn on both sludge and control farms (Table 11-22). The amounts of time spent in field work on sludge and control farms were similar, 9.1 and 10.9 hours per week respectively.

The amount of time spent in livestock work was approximately one-half the amount of time spent in field work (Table 11-22). Unlike the field work, the livestock work was distributed fairly evenly throughout the year in all counties (Tables 11-23 - 11-25). The amount of livestock work for the combined 3 locations was similar in the sludge and control groups (Table 11-22).

The control farm participants spent more hours off-farm than did the sludge farm participants, 47.3 vs. 39.5 respectively. This difference was consistent in Medina and Franklin-Pickaway counties, but not in Clark County (Tables 11-23 - 11-25). The large difference observed in Franklin-Pickaway counties is largely due to the large number of field laborers at the plant nursery.

Home Produced Food Consumed

A higher percent of meat consumed by the sludge farm residents was home raised than for the control farm residents (Table 11-26). This difference was contributed by participants mainly from Franklin-Pickaway counties (Tables 11-27 - 11-29); however, it was not statistically significant using the analysis of variance.

For fruit and vegetable consumption, the percent that was home grown was similar in sludge and control groups (Tables 11-26 - 11-29). The percent of fruits and vegetables that was home grown was highest for Medina County, followed by Franklin-Pickaway County and then Clark County.

By analysis of variance there was a significant season effect on the percent of home produced meat and fruits-vegetables consumed ($P < 0.05$). The differences between counties described above were not statistically significant.

TUBERCULIN TESTING

A total if 153 and 119 tuberculin tine tests were administered, prior to application of sludge, in the sludge and control groups, respectively (Table 11-30). In the sludge group, seven persons had significant reactions of 5 mm or greater induration at the site of inoculation in the pre-sludge application baseline test. Six of these were followed with a Mantoux intradermal skin test. Of these, 2 persons had significant reactions. Both of these persons then received a chest X-ray and both were negative radiographically (Tables 11-31 - 11-33).

In the control group, 5 of 119 persons tested before sludge application had significant tine test reactions (Table 11-30). Two of these were followed with a Mantoux test and none of these were significant.

In Medina County, one person was positive on the tine test administered after the first sludge application (Table 11-31). This person was also positive on the pre-sludge tine test and did not receive the second and third annual test.

In Franklin-Pickaway Counties, 2 previously negative persons in the sludge group had significant tine tests in the second post sludge application year (Table 11-32). One of the individuals was not given the Mantoux test at the request of the family physician. The other person received a Mantoux test which was negative.

HUMAN ILLNESSES

Illness Rates

The number of persons ill, number of new acute illness and overall illness rates for each county are presented in Table 11-34. Chronic illnesses, reported in pre-sludge interviews, were not included. Individuals reporting an illness with similar symptoms in 2 consecutive interviews were further questioned about the time lapse between the two illnesses. An asymptomatic period of at least one week between the illnesses was required to consider them separate episodes. The illness rates were higher on the control farms than on the sludge farms in Medina County and Franklin-Pickaway Counties, but not in Clark County where the sludge farms had higher rates.

Illness rates per 100 person-years at risk for specific symptoms were calculated for all 3 study locations (Tables 11-35 - 11-38). For each symptom category in Medina County and in Franklin-Pickaway Counties the rates were higher on the control farms than on the sludge farms. The opposite relationship existed in Clark County where the sludge farm rates were higher.

Illness rates were also calculated for specific age and sex groups. The overall rates for all illnesses are shown in Figure 11-4. The rates for both sludge and control farms are highest in the younger ages (13 years). They then drop in the 13-19 year age group followed by a rise in the 20-59 years. The male- and female-specific rates are similar in the sludge and control groups except for an abnormally high female rate in the 20-29 year age group for control respondents.

The respiratory illness rates are shown in Figure 11-5. Again the rates for sludge and control respondents were very similar except for the higher female rate in the 20-29 year age group for controls. The digestive illness rates (Figure 11-6) were very similar in the sludge and control groups and did not reveal the higher rate in females on control farms observed for respiratory illnesses.

Statistical Analysis

Following descriptive examination of the data, the matched pair logistic regression analysis was performed. The health history information was statistically examined separately for the 3 month time period following the first sludge application and for the corresponding time period following the second sludge application. There were only a few farms that received a third sludge application, too few for statistical analysis. The corresponding odds ratios and P values for each symptom and for combined symptoms reported on sludge and control farms are shown in Tables 11-39 - 11-40, respectively. There were no statistically significant associations between sludge use and the occurrence among farm residents of any single symptom or any of the 4 combinations of symptoms.

Consistency of Reporting

If sludge applications were responsible for human illnesses then the numbers of illnesses in the post-sludge period should be greater than the number in the pre-sludge period. On the other hand, if the respondents became conditioned by the telephone interviews every month, then they might tend to increasingly underreport as the study progressed. In Tables 11-41 - 11-44, the numbers of reported illness in the pre-sludge period and after each sludge application are presented. There was no pattern of increasing or decreasing reporting over time for any of the symptoms. On the average, the percentage of persons reporting any illnesses in the post-sludge period was similar to the percentage reporting illness in the pre-sludge period. There were too few reported illness due to specific symptoms in some counties to allow meaningful analysis and interpretation.

Amount of Sludge Exposure

By determining the number of hours of sludge exposure per week and the illness rates for various levels of exposure, an examination for a dose response relationship was possible (Table 11-45). Contrary to the hypothesis of more illness with greater sludge exposure (dose), the illness rate was highest (314.5) for persons with no sludge exposure. The rate for the highest weekly sludge exposure, 1 1/2 hours, was 230.2. A multiple regression analysis was conducted of the proportion of persons with any symptom in sludge farm families as a function of exposure dose (number of tons per acre multiplied by number of hours spent in sludge applied fields per week), proportion of school age children per family, number of interviews and number of people per family. As apparent from the descriptive data in Table 11-45, the multiple regression analysis confirmed a negative relationship between exposure dose and illness; however, this was not significant at the 5% level. The proportion of school age children and number of people per family were also nonsignificant independent variables. As one would expect, the more interviews conducted, the larger the proportion ill in a family. This relationship approached significance, $P = 0.07$. The tendency of lower risk of illness in those farm members who work in the fields as compared to the illness risk in persons not working on the fields, such as the housewives and children, was not observed on the control farms.

Relationship Between Illness and Seroconversion

The results of the viral and serological findings are reported in Section 15. The seroconversions that could have been due to sludge exposure were identified for the sludge and control farms as explained in the Methods. There were 15 seroconversions on sludge farms and 14 seroconversions on control farms (Table 11-46). The distributions of Coxsackie A, Coxsackie B and Echovirus infections on the sludge and control farms were similar.

The distributions of respiratory symptoms and digestive symptoms associated with the seroconversions on sludge and control farms were similar (Table 11-46). The seroconversions on the sludge farms were associated with 5 visits to the physician while on the control farms none of the persons with seroconversions visited the physician. However, the worst illness status experienced by the ill persons were similar in the 2 groups (Table 11-47). Most of the infections resulted in person feeling ill and either continuing to work or staying at home. Fewer persons responded that they were confined to a bed or to the hospital. A relatively large number of seroconversions did not result in self-perceived illness and none were associated with death.

ANIMAL POPULATION CHARACTERISTICS

Species and Type of Operation

The numbers of animal units of various species and types of operations on sludge and control farms are shown in Tables 11-48 - 11-51. Cattle raising was the most common type of livestock enterprise followed by swine, equine, avian and porcine production. Within study locations, there were differences between sludge and control farms in terms of the numbers of units of certain species or types of operation (Tables 11-49 - 11-51). However when these data were combined, the sludge and control groups were relatively well balanced (Table 11-48). The frequency distribution of unit-years at risk and animal-years at risk for various species were also similar in the sludge and control groups.

Distribution of Hours on Fields and Sludge Exposure

Among the livestock species, horses and sheep spent the most hours on fields on both sludge and control farms followed by cattle, avian species, and swine (Tables 11-52 - 11-55). Cats spent more time on the fields than did the dogs. On the sludge farms, the cattle and sheep in Franklin-Pickaway Counties had the most exposure to sludge treated fields. The only species exposed to sludge treated fields in Medina County were cats, dogs, horses and avian species (Table 11-53). In Clark County, cattle, swine, dogs, cats and avian species were exposed (Table 11-55).

Consumption of Home Grown Feed

Except for dogs and cats, the farm animals' ration was primarily comprised of home raised feedstuffs (Table 11-52). This pattern was similar in both sludge and control groups and in all 3 study locations (Tables 11-53 - 11-55).

ANIMAL ILLNESSES

Bovine

The numbers of bovine production units and cattle that became ill with specific signs and their respective illness rates for sludge and control groups are presented in Tables 11-56 - 11-59. Both the unit based and animal based illness rates were descriptively higher in the control than in the sludge groups. The most common signs in the sludge and control groups were: off feed, weakness, and nasal discharge. The signs were statistically analyzed separately and in respiratory (cough, nasal discharge and difficult breathing) and digestive (constipation, diarrhea and fecal blood) groupings. None of the observed differences between sludge and control farms with numbers sufficient for testing were statistically significant.

Porcine

The numbers of porcine production units and individual animals that became ill with specific signs and their respective illness rates for sludge and control groups are presented in Tables 11-60 - 11-63. As for the bovine species, both the unit and animal illness rates were descriptively higher in the control than in the sludge groups. The most common conditions in the sludge and control groups were: weakness, diarrhea, off feed, difficult breathing, cough, and sudden death. None of the observed differences between sludge and control farms for specific signs and for respiratory and digestive groupings were statistically significant.

Ovine

The numbers of ovine production units and sheep that became ill with specific signs and their respective illness rates for sludge and control groups are presented in Tables 11-64 - 11-67. Both the unit and animal illness rates were again higher on the control farms than on the sludge farms. The most common conditions in the sludge and control groups were: weakness, off feed, difficult breathing, fever and sudden death. None of the observed differences between sludge and control farms for specific signs and for the respiratory and digestive groupings were statistically significant.

Equine

The numbers of equine production units and equines that became ill with specific signs and their respective illness rates on sludge and control farms are presented in Tables 11-68 - 11-71. Both the unit and animal illness rates were higher in the control than in the sludge groups. There were very small numbers of illnesses; therefore, no tests of significance were performed.

Avian

The numbers of avian production units and individual birds that became ill with specific signs and their respective illness rates for sludge and control farms are presented in Table 11-72 - 11-75. Both the unit and animal illness rates were higher in the control than in the sludge groups. The most common conditions in the sludge and control groups were sudden death and weakness. None of the observed differences between sludge and control groups were statistically significant.

Canine

The numbers of canine units and individual dogs that became ill with specific illness and their respective illness rates for sludge and control groups are presented in Tables 11-76 - 11-79. Again the pattern of higher unit and animal illness rates in the control group than in the sludge group. The most common symptoms were off feed, fever and weakness. None of the observed differences were statistically significant.

Feline

The numbers of feline units and individual cats that became ill with specific symptoms and their respective illness rates for sludge and control groups are presented in Table 11-80 - 11-83. The overall unit illness rate was higher in the control group but the overall animal illness rate was higher in the sludge group. The most common symptoms were weakness, fever, and difficult breathing. None of the observed differences were statistically significant.

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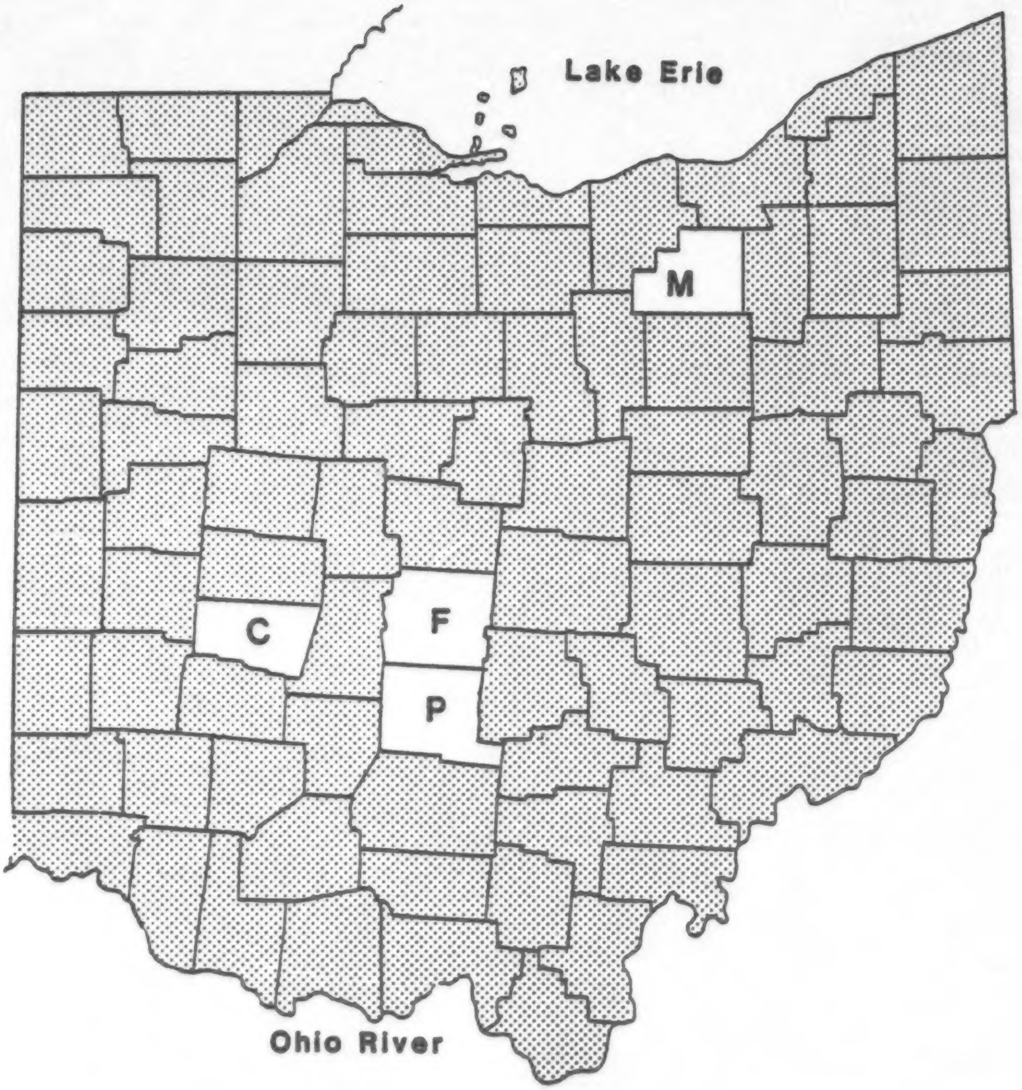
Lake Erie
M
C
F
P
Ohio River
COUNTIES: Clark = C Medina = M
Franklin = F Pickaway = P

Figure 11-1. Location of counties with participating sewage treatment facilities and farms.

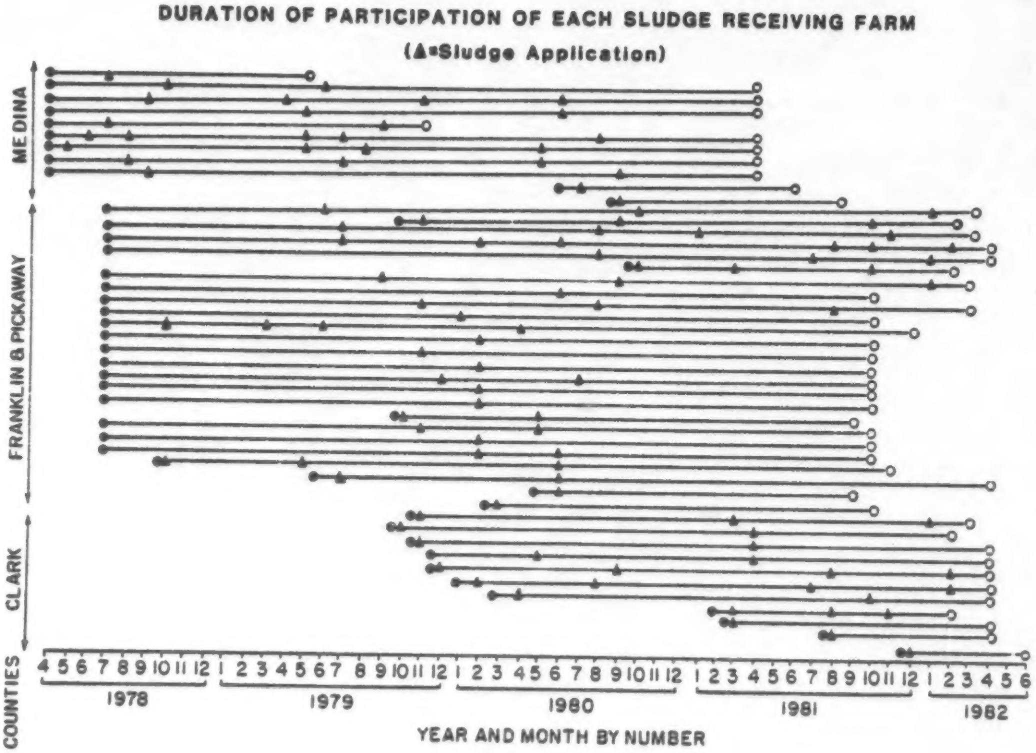

Figure 11-2. Duration of participation of each sludge receiving farm, by county and date of sludge application; ● = dates of first interview; ▲= dates of sludge application; ○ = dates of final interview.

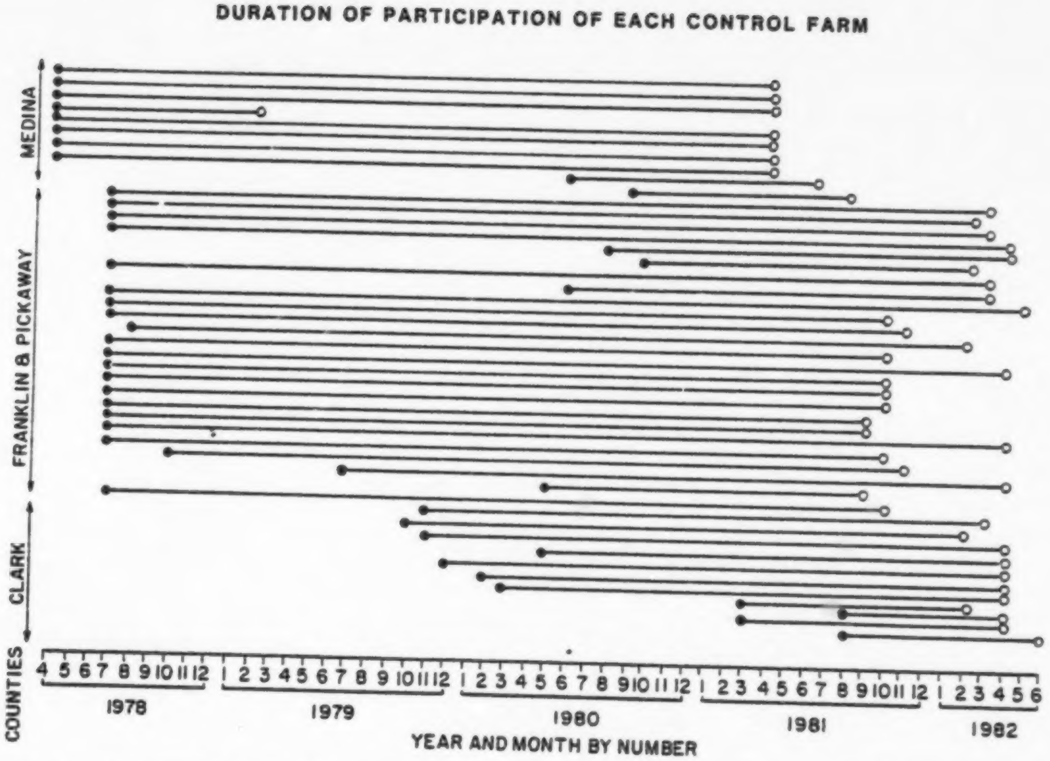

Figure 11-3. Duration of participation of each control farm by county; ●= dates of first interview; ○ = dates of final interview.

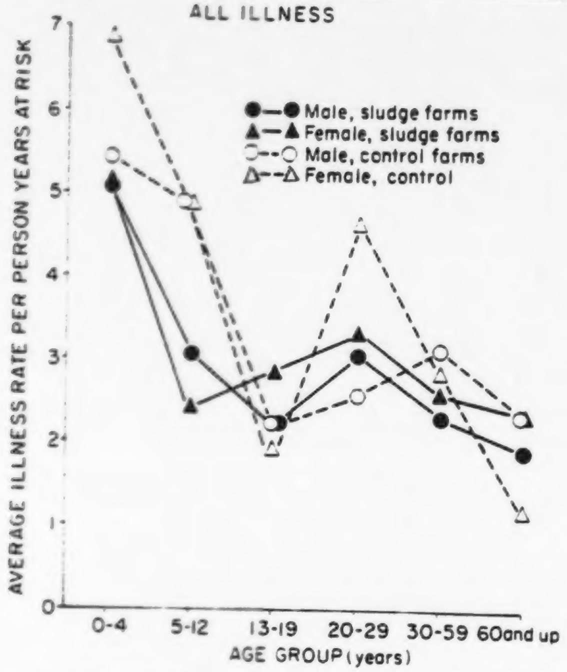

Figure 11-4. Age and sex-specific rates, for all illnesses among persons on sludge and control farms, ●—● males, sludge farms: ▲—▲ females, sludge farms: ○—○ males, control farms: △—△ females, control farms.

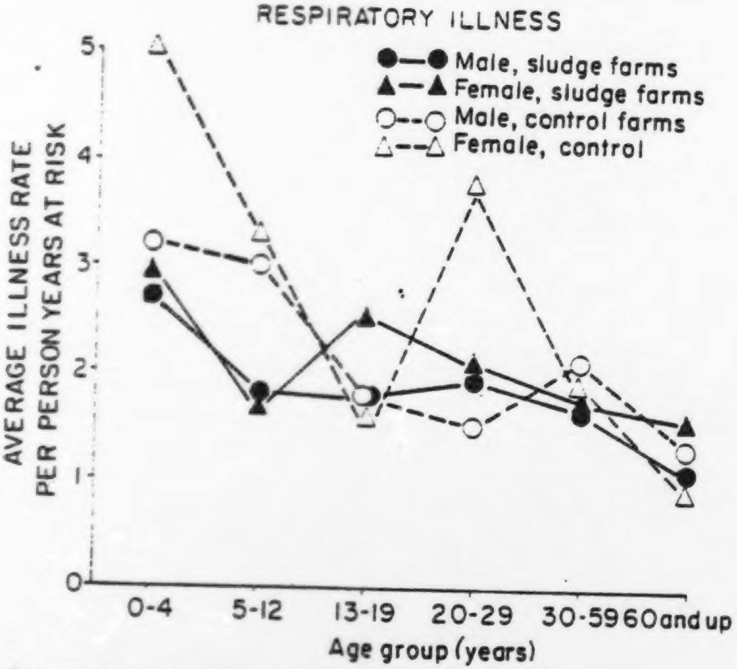

Figure 11-5. Age and sex-specific respiratory illness rates for persons on sludge and control farms; ●—● males, sludge farms: ▲—▲ females, sludge farms; ○—○ males, control farms; △—△ females, control farms.

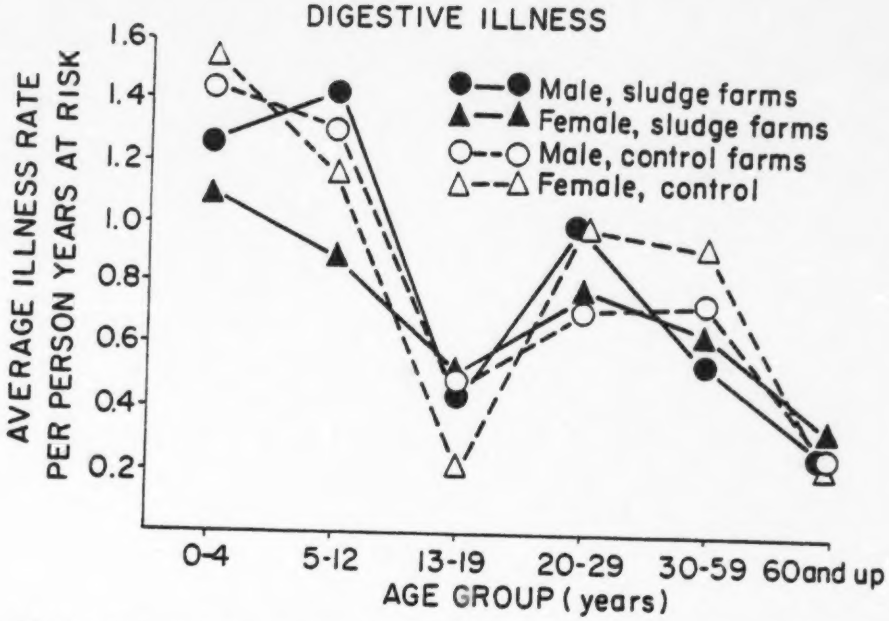

Figure 11-6. Age and sex-specific digestive illness rates for persons on sludge and control farms; ●–● males, sludge farms; ▲–▲ females, sludge farms; ○–○ males, control farms; △–△ females, control farms.

TABLE 11-1. NUMBER OF FARMS AND PARTICIPANTS IN SLUDGE AND CONTROL GROUPS BY YEARS OF PARTICIPATION, ALL COUNTIES

Unit	Study Group	Number Started	Number of Participating Year(s)[a] 1	2	3
Farms	Sludge	47	47	36	13
	Control	46	46	37	13
Families	Sludge	78	77	56	21
	Control	53	53	40	13
Eligible Persons[b]	Sludge	200	200	153	59
	Control	174	174	124	44
Participants	Sludge	165	165	126	53
	Control	130	130	109	37

[a] Participation calculated from the date of first sludge application for each sludge farm; for each control farm participation was calculated from the date of interview closest to the date of first sludge application on the corresponding sludge farm.

[b] A person eligible to participate was one who resided on or regularly visited (in the case of sludge receiving farms, residence or visits must not be on a site remote from sludge) a designated project farm.

TABLE 11-2. NUMBER OF FARMS AND PARTICIPANTS IN SLUDGE AND CONTROL GROUPS BY YEARS OF PARTICIPATION, MEDINA COUNTY

Unit	Study Group	Number Started	Number of Participating Year(s)[a] 1	2	3
Farms	Sludge	11	11	7	6
	Control	10	10	7	6
Families	Sludge	12	12	7	6
	Control	10	10	7	6
Eligible Persons[b]	Sludge	37	37	23	19
	Control	33	33	23	21
Participants	Sludge	31	31	20	18
	Control	26	26	21	19

[a] Participation calculated from the date of first sludge application for each sludge farm; for each control farm participation was calculated from the date of interview closest to the date of first sludge application on the corresponding sludge farm.

[b] A person eligible to participate was one who resided on or regularly visited (in the case of sludge receiving farms, residence or visits must not be on a site remote from sludge) a designated project farm.

TABLE 11-3. NUMBER OF FARMS AND PARTICIPANTS IN SLUDGE AND CONTROL GROUPS BY YEARS OF PARTICIPATION, FRANKLIN AND PICKAWAY COUNTIES

Unit	Study Group	Number Started	Number of Participating Year(s)[a] 1	2	3
Farms	Sludge	25	25	22	7
	Control	25	25	24	7
Families	Sludge	47	46	39	15
	Control	28	28	27	7
Eligible Persons[b]	Sludge	116	116	105	40
	Control	87	87	83	23
Participants	Sludge	101	99	86	35
	Control	76	76	72	18

[a] Participation calculated from the date of first sludge application for each sludge farm; for each control farm participation was calculated from the date of interview closest to the date of first sludge application on the corresponding sludge farm.

[b] A person eligible to participate was one who resided on or regularly visited (in the case of sludge receiving farms, residence or visits must not be on a site remote from sludge) a designated project farm.

TABLE 11-4. NUMBER OF FARMS AND PARTICIPANTS IN SLUDGE AND CONTROL GROUPS BY YEARS OF PARTICIPATION, CLARK COUNTY

Unit	Study Group	Number Started	Number of Participating Year(s)[a] 1	2	3
Farms	Sludge	11	11	7	--[c]
	Control	11	11	6	--
Families	Sludge	19	19	10	--
	Control	15	15	6	--
Eligible Persons[b]	Sludge	47	47	25	--
	Control	54	54	18	--
Participants	Sludge	32	35	20	--
	Control	28	28	16	--

[a] Participation calculated from the date of first sludge application for each sludge farm; for each control farm participation was calculated from the date of interview closest to the date of first sludge application on the corresponding sludge farm.

[b] A person eligible to participate was one who resided on or regularly visited (in the case of sludge receiving farms, residence or visits must not be on a site remote from sludge) a designated project farm.

[c] Blank indicates that none of the participants were in the project for the third year because of late recruitment.

TABLE 11-5. DISTRIBUTION OF POPULATION IN SLUDGE AND CONTROL GROUPS BY AGE AND SEX, ALL COUNTIES

Age (yr) and Sex (M&F)	Sludge			Control		
	Total	No. of Each Sex	Percent	Total	No. of Each Sex	Percent
All ages	165		100.0[a]	130		100.0
M		97			73	
F		68			57	
0 - 9	17		10.3	13		10.0
M		7			7	
F		10			6	
10-19	22		13.3	19		14.6
M		14			12	
F		8			7	
20-29	24		14.5	12		9.2
M		15			8	
F		9			4	
30-39	24		14.5	24		18.5
M		14			14	
F		10			10	
40-49	24		14.5	10		7.7
M		13			6	
F		11			4	
50-59	28		17.0	27		20.8
M		19			12	
F		9			15	
60-69	16		9.7	23		17.7
M		9			13	
F		7			10	
70 and over	10		6.1	2		1.5
M		6			1	
F		4			1	

[a] Column does not add to 100.0% due to rounding.

TABLE 11-6. DISTRIBUTION OF POPULATION IN SLUDGE AND CONTROL GROUPS BY AGE AND SEX, MEDINA COUNTY

Age (yr) and Sex (M&F)	Sludge			Control		
	Total	No. of Each Sex	Percent	Total	No. of Each Sex	Percent
All ages	31		100.0[a]	26		100.0[a]
M		15			13	
F		16			13	
0 - 9	4		12.9	5		19.2
M		1			3	
F		3			2	
10-19	4		12.9	3		11.5
M		4			2	
F		0			1	
20-29	1		3.2	1		3.8
M		1			1	
F		0			0	
30-39	4		12.9	5		19.2
M		2			2	
F		2			3	
40-49	4		12.9	2		7.7
M		2			1	
F		2			1	
50-59	9		29.0	7		26.9
M		4			3	
F		5			4	
60-69	5		16.1	3		11.5
M		2			1	
F		3			2	
70 and over	0		0	0		0
M		0			0	
F		0			0	

[a] Column does not add to 100.0% due to rounding.

TABLE 11-7. DISTRIBUTION OF POPULATION IN SLUDGE AND CONTROL GROUPS BY AGE AND SEX, FRANKLIN AND PICKAWAY COUNTIES

Age (yr) and Sex (M&F)	Sludge			Control		
	Total	No. of Each Sex	Percent	Total	No. of Each Sex	Percent
All ages	101		100.0	76		100.0[a]
M		59			41	
F		42			35	
0 - 9	10		9.9	8		10.5
M		4			4	
F		6			4	
10-19	15		14.9	13		17.1
M		9			7	
F		6			6	
20-29	12		11.9	8		10.5
M		7			4	
F		5			4	
30-39	16		15.8	12		15.8
M		9			7	
F		7			5	
40-49	18		17.8	8		10.5
M		10			5	
F		8			3	
50-59	11		10.9	13		17.1
M		9			5	
F		2			8	
60-69	10		9.9	12		15.8
M		5			8	
F		5			4	
70 and over	9		8.9	2		2.6
M		6			1	
F		3			1	

[a] Column does not add to 100.0% due to rounding.

TABLE 11-8. DISTRIBUTION OF POPULATION IN SLUDGE AND CONTROL GROUPS BY AGE AND SEX, CLARK COUNTY

Age (yr) and Sex (M&F)	Sludge			Control		
	Total	No. of Each Sex	Percent	Total	No. of Each Sex	Percent
All ages	36		100.0[a]	28		100.0
M		23			19	
F		13			9	
0 - 9	4		11.1	0		0.0
M		2			0	
F		2			0	
10-19	3		8.3	3		10.7
M		1			3	
F		2			0	
20-29	11		30.6	3		10.7
M		7			3	
F		4			0	
30-39	5		13.9	7		25.0
M		4			5	
F		1			2	
40-49	2		5.6	0		0.0
M		1			0	
F		1			0	
50-59	8		22.2	7		25.0
M		6			4	
F		2			3	
60-69	2		5.6	8		28.6
M		2			4	
F		0			4	
70 and over	1		2.8	0		0.0
M		0			0	
F		1			0	

[a] Column does not add to 100.0% due to rounding.

TABLE 11-9. DISTRIBUTION OF TOTAL RURAL FARM POPULATION BY AGE AND SEX (1970 CENSUS), ALL COUNTIES

Age (yr) and Sex (M&F)	Total	Number of Each Sex	Percent
All ages	23,877		100.0
M		12,040	
F		11,837	
0 - 9	3,884		16.3
M		2,003	
F		1,881	
10-19	4,976		20.8
M		2,602	
F		2,374	
20-29	2,137		9.0
M		1,052	
F		1,085	
30-39	2,499		10.5
M		1,237	
F		1,262	
40-49	2,982		12.5
M		1,430	
F		1,552	
50-59	3,300		13.8
M		1,668	
F		1,632	
60-69	2,186		9.1
M		1,184	
F		1,002	
70 and over	1,913		8.0
M		864	
F		1,049	

TABLE 11-10. DISTRIBUTION OF TOTAL RURAL FARM POPULATION BY AGE AND SEX (1970 CENSUS), MEDINA COUNTY

Age (yr) and Sex (M&F)	Total	Number of Each Sex	Percent
All ages	7,109		100.0[a]
M		3,653	
F		3,456	
0 - 9	1,238		17.4
M		591	
F		647	
10-19	1,585		22.3
M		913	
F		672	
20-29	663		9.3
M		324	
F		339	
30-39	753		10.6
M		366	
F		387	
40-49	883		12.4
M		442	
F		441	
50-59	877		12.3
M		443	
F		434	
60-69	631		8.9
M		330	
F		301	
70 and over	479		6.7
M		244	
F		235	

[a] Column does not add to 100.0% due to rounding.

TABLE 11-11. DISTRIBUTION OF TOTAL RURAL FARM POPULATION BY AGE AND SEX (1970 CENSUS), FRANKLIN AND PICKAWAY COUNTIES

Age (yr) and Sex (M&F)	Total	Number of Each Sex	Percent
All ages	10,247		100.0[a]
M		5,089	
F		5,158	
0 - 9	1,581		15.4
M		862	
F		719	
10-19	2,205		21.5
M		1,114	
F		1,091	
20-29	888		8.6
M		409	
F		479	
30-39	1,057		10.3
M		517	
F		540	
40-49	1,287		12.6
M		635	
F		652	
50-59	1,407		13.7
M		660	
F		747	
60-69	946		9.2
M		534	
F		412	
70 and over	876		8.5
M		358	
F		518	

[a] Column does not add to 100% due to rounding.

TABLE 11-12. DISTRIBUTION OF TOTAL RURAL FARM POPULATION BY AGE AND SEX (1970 CENSUS), CLARK COUNTY

Age (yr) and Sex (M&F)	Total	Number of Each Sex	Percent
All ages	6,521		100.0
M		3,298	
F		3,223	
0 - 9	1,065		16.3
M		550	
F		515	
10-19	1,186		18.2
M		575	
F		611	
20-29	586		9.0
M		319	
F		267	
30-39	689		10.6
M		354	
F		335	
40-49	812		12.4
M		353	
F		459	
50-59	1,016		15.6
M		565	
F		451	
60-69	609		9.3
M		320	
F		289	
70 and over	558		8.6
M		262	
F		296	

TABLE 11-13. NUMBER OF PERSONS ON EACH FARM OR IN EACH FAMILY OF THE SLUDGE RECEIVING AND CONTROL GROUPS, ALL COUNTIES

Size of Family (No. of Persons)	Sludge		Control	
	Farms	Families	Farms	Families
Total	46	78	46	53
1	4	31	7	12
2	16	28	22	24
3	7	4	3	6
4	5	9	6	5
5	3	4	4	4
6	7	2	3	2
7	3	0	0	0
8	0	0	1	0
9	1	0	0	0

TABLE 11-14. NUMBER OF PERSONS ON EACH FARM OR IN EACH FAMILY OF THE SLUDGE RECEIVING AND CONTROL GROUPS, MEDINA COUNTY

Size of Family (No. of Persons)	Sludge		Control	
	Farms	Families	Farms	Families
Total	11	12	10	10
1	2	2	2	2
2	5	6	5	5
3	1	1	0	0
4	1	2	1	1
5	0	0	2	2
6	2	1	0	0

TABLE 11-15. NUMBER OF PERSONS ON EACH FARM OR IN EACH FAMILY OF THE SLUDGE RECEIVING AND CONTROL GROUPS, FRANKLIN AND PICKAWAY COUNTIES

Size of Family (No. of Persons)	Sludge		Control	
	Farms	Families	Farms	Families
Total	24	47	25	28
1	1	18	4	4
2	7	18	10	11
3	4	2	2	6
4	2	5	4	4
5	3	3	2	2
6	4	1	2	1
7	2	0	0	0
8	0	0	1	0
9	1	0	0	0

TABLE 11-16. NUMBER OF PERSONS ON EACH FARM OR IN EACH FAMILY OF THE SLUDGE RECEIVING AND CONTROL GROUPS, CLARK COUNTY

Size of Family (No. of Persons)	Sludge		Control	
	Farms	Families	Farms	Families
Total	11	19	11	15
1	1	11	1	6
2	4	4	7	8
3	2	1	1	0
4	2	2	1	0
5	0	1	0	0
6	1	0	1	1
7	1	0	0	0

TABLE 11-17. DISTRIBUTION OF STUDY POPULATIONS BY SEX AND CIGARETTE SMOKING STATUS AT THE TIME OF FINAL INTERVIEW, ALL COUNTIES

County	Sex and Smoking Status	Sludge	Percent	Control	Percent
All Counties		168	100.0	130	100.0
	All smokers[a]	47	28.0	35	26.9
	All non Smokers[b]	121	72.0	95	73.1
	Male	97	100.0	73	100.0
	Smokers	32	33.0	23	31.5
	Non Smokers	65	67.0	50	68.5
	Female	71	100.0	57	100.0
	Smokers	15	21.1	12	21.1
	Non Smokers	56	78.9	45	78.9
Medina	Male	15	100.0	13	100.0
	Smokers	3	20.0	3	23.1
	Non Smokers	12	80.0	10	76.9
	Female	16	100.0	13	100.0
	Smokers	5	31.3	1	7.7
	Non Smokers	11	68.7	12	92.3
Franklin - Pickaway	Male	59	100.0	41	100.0
	Smokers	24	40.7	15	36.6
	Non Smokers	35	59.3	26	63.4
	Female	42	100.0	35	100.0
	Smokers	9	21.4	9	25.7
	Non Smokers	33	78.6	26	74.3
Clark	Male	23	100.0	19	100.0
	Smokers	5	21.7	5	26.3
	Non Smokers	18	78.3	14	73.7
	Female	13	100.0	9	100.0
	Smokers	1	7.7	2	22.2
	Non Smokers	12	92.3	7	77.8

[a] Individuals who smoked cigarettes at any time before the final interview.

[b] Individuals who never smoked cigarettes before the final interview.

TABLE 11-18. DISEASE AND IMMUNIZATION HISTORY OF PERSONS IN SLUDGE AND CONTROL GROUPS AT THE TIME OF INITIAL INTERVIEW, ALL COUNTIES

Disease	Response	Sludge		Control	
		Number	Percent	Number	Percent
Polio	All Responses	168	100.0	130	100.0
	Previously Had Disease	1	0.6	0	0.0
	Did Not Have Disease				
	Immunized	150	89.3	120	92.3
	Not Immunized	17	10.1	9	6.9
	Unknown Status	0	0.0	1	0.8
Measles	All Responses	168	100.0	130	100.0
	Previously Had Disease	90	53.6	74	56.9
	Did Not Have Disease				
	Immunized	47	28.0	33	25.4
	Not Immunized	16	9.5	16	12.3
	Unknown Status	15	8.9	7	5.4
Rubella	All Responses	168	100.0	130	100.0
	Previously Had Disease	98	58.3	77	59.2
	Did Not Have Disease				
	Immunized	49	29.2	30	23.1
	Not Immunized	8	4.8	9	6.9
	Unknown Status	13	7.7	14	10.8

TABLE 11-19. DISEASE AND IMMUNIZATION HISTORY OF PERSONS IN SLUDGE AND CONTROL GROUPS AT THE TIME OF INITIAL INTERVIEW, MEDINA COUNTY

Disease	Response	Sludge		Control	
		Number	Percent	Number	Percent
Polio	All Responses	31	100.0	26	100.0
	Previously Had Disease	1	3.2	0	0.0
	Did Not Have Disease				
	Immunized	23	74.2	21	80.8
	Not Immunized	7	22.6	5	19.2
	Unknown Status	0		0	
Measles	All Responses	31	100.0	26	100.0
	Previously Had Disease	11	35.5	13	50.0
	Did Not Have Disease				
	Immunized	7	22.6	7	26.9
	Not Immunized	8	25.8	4	15.4
	Unknown Status	5	16.1	2	7.7
Rubella	All Responses	31	100.0	26	100.0
	Previously Had Disease	18	58.0	16	61.5
	Did Not Have Disease				
	Immunized	11	35.5	6	23.1
	Not Immunized	0	0.0	1	3.9
	Unknown Status	2	6.5	3	11.5

TABLE 11-20. DISEASE AND IMMUNIZATION HISTORY OF PERSONS IN SLUDGE AND CONTROL GROUPS AT THE TIME OF INITIAL INTERVIEW, FRANKLIN AND PICKAWAY COUNTIES

Disease	Response	Sludge		Control	
		Number	Percent	Number	Percent
Polio	All Responses	101	100.0	76	100.0
	Previously Had Disease	0	0.0	0	0.0
	Did Not Have Disease				
	Immunized	91	90.1	71	93.4
	Not Immunized	10	9.9	4	5.3
	Unknown Status	0	0.0	1	1.3
Measles	All Responses	101	100.0	76	100.0
	Previously Had Disease	57	56.5	39	51.3
	Did Not Have Disease				
	Immunized	30	29.7	21	27.6
	Not Immunized	7	6.9	12	15.8
	Unknown Status	7	6.9	4	5.3
Rubella	All Responses	101	100.0	76	100.0
	Previously Had Disease	57	56.5	42	55.3
	Did Not Have Disease				
	Immunized	30	29.7	19	25.0
	Not Immunized	7	6.9	6	7.9
	Unknown Status	7	6.9	9	11.8

TABLE 11-21. DISEASE AND IMMUNIZATION HISTORY OF PERSONS IN SLUDGE AND CONTROL GROUPS AT THE TIME OF INITIAL INTERVIEW, CLARK COUNTY

Disease	Response	Sludge		Control	
		Number	Percent	Number	Percent
Polio	All Responses	36	100.0	28	100.0
	Previously Had Disease	0	0.0	0	0.0
	Did Not Have Disease				
	Immunized	36	100.0	28	100.0
	Not Immunized	0		0	
	Unknown Status	0		0	
Measles	All Responses	36	100.0	28	100.0
	Previously Had Disease	22	61.1	22	78.6
	Did Not Have Disease				
	Immunized	10	27.8	5	17.8
	Not Immunized	1	2.8	0	0.0
	Unknown Status	3	8.3	1	3.6
Rubella	All Responses	36	100.0	28	100.0
	Previously Had Disease	23	63.9	19	67.9
	Did Not Have Disease				
	Immunized	8	22.2	5	17.9
	Not Immunized	1	2.8	2	7.1
	Unknown Status	4	11.1	2	7.1

TABLE 11-22. SEASONAL ON-AND OFF-FARM WORK[a] (AVERAGE NO. OF HOURS PER WEEK) PROFILES OF CONTROL AND SLUDGE POPULATIONS IN ALL COUNTIES

Type of Time	Sludge				
	All Seasons	Winter	Spring	Summer	Autumn
Total Hours per Week	168.0	168.0	168.0	168.0	168.0
Field Work	9.1	2.1	11.3	10.8	12.4
On Sludge Land	1.2	0.4	1.5	1.5	1.5
Livestock Work	4.6	4.8	4.7	5.0	3.9
Off-Farm Time	39.5	41.2	37.1	39.8	40.2
School	4.9	5.6	5.5	1.7	6.9
Job	10.4	10.1	10.4	10.1	11.1
Other	24.2	25.5	21.2	28.0	22.2
	Control				
Total Hours per Week	168.0	168.0	168.0	168.0	168.0
Field Work	10.9	3.2	12.5	11.7	16.0
On Sludge Land[b]	-----	-----	-----	-----	-----
Livestock Work	4.8	5.7	4.8	4.5	4.4
Off-Farm Time	47.3	48.1	47.5	46.8	46.5
School	4.6	5.2	5.2	1.8	6.2
Job	9.5	9.8	9.4	8.9	9.8
Other	33.2	33.1	32.9	36.1	30.5

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[a] Number of hours per week was calculated by averaging the monthly responses for each season over the three year period, beginning the first monthly interview following the initial sludge application and the corresponding monthly interview for controls. Winter = Jan., Feb., March; Spring = April, May, June; Summer = July, Aug., Sept.; Autumn = Oct., Nov., Dec.

[b] Does not apply to control farms.

TABLE 11-23. SEASONAL ON-AND OFF-FARM WORK[a] (AVERAGE NO. OF HOURS PER WEEK) PROFILES OF CONTROL AND SLUDGE POPULATIONS IN MEDINA COUNTY

Type of Time	Sludge				
	All Seasons	Winter	Spring	Summer	Autumn
Total Hours per Week	168.0	168.0	168.0	168.0	168.0
Field Work	5.2	1.7	6.0	9.2	4.7
On Sludge Land[b]	0.8	0.2	1.4	1.1	0.5
Livestock Work	3.3	3.5	3.1	3.2	3.5
Off-Farm Time	43.0	44.7	40.1	43.1	44.0
School	6.8	7.9	7.8	2.8	8.7
Job	13.3	13.2	13.5	12.3	14.3
Other	22.9	23.6	18.8	28.0	21.0
	Control				
Total Hours per Week	168.0	168.0	168.0	168.0	168.0
Field Work	10.2	2.6	9.7	14.8	13.4
On Sludge Land[b]	-----	-----	-----	-----	-----
Livestock Work	5.7	6.5	5.8	5.2	5.1
Off-Farm Time	55.6	60.8	54.3	54.7	52.2
School	5.2	5.5	6.7	2.4	6.2
Job	11.1	12.0	10.6	9.0	12.6
Other	39.3	43.3	37.0	43.3	33.4

[a] Number of hours per week was calculated by averaging the monthly responses for each season over the three year period, beginning the first monthly interview following the initial sludge application and the corresponding monthly interview for controls. Winter = Jan., Feb., March; Spring = April, May, June; Summer = July, Aug., Sept.; Autumn = Oct., Nov., Dec.

[b] Does not apply to control farms.

TABLE 11-24. SEASONAL ON-AND OFF-FARM WORK[a] (AVERAGE NO. OF HOURS PER WEEK) PROFILES OF CONTROL AND SLUDGE POPULATIONS IN FRANKLIN-PICKAWAY COUNTIES

Type of Time	Sludge				
	All Seasons	Winter	Spring	Summer	Autumn
Total Hours per Week	168.0	168.0	168.0	168.0	168.0
Field Work	10.3	2.4	12.1	12.2	14.6
On Sludge Land[b]	1.6	0.6	1.8	1.9	2.0
Livestock Work	3.9	3.8	3.8	4.6	3.4
Off-Farm Time	38.5	41.2	37.0	39.0	37.2
School	5.0	5.8	5.6	1.8	7.0
Job	9.7	9.6	10.0	9.7	9.6
Other	23.8	25.8	21.4	27.5	20.6
	Control				
Total Hours per Week	168.0	168.0	168.0	168.0	168.0
Field Work	9.6	3.2	11.3	9.8	13.8
On Sludge Land[b]	-----	-----	-----	-----	-----
Livestock Work	4.2	4.9	4.0	4.2	3.6
Off-Farm Time	47.6	47.1	48.9	47.2	47.0
School	5.5	6.4	6.1	2.0	7.5
Job	10.6	10.9	10.4	10.4	10.6
Other	31.5	29.8	32.4	34.8	28.9

[a] Number of hours per week was calculated by averaging the monthly responses for each season over the three year period, beginning the first monthly interview following the initial sludge application and the corresponding monthly interview for controls. Winter = Jan., Feb., March; Spring = April, May, June; Summer = July, Aug., Sept.; Autumn = Oct., Nov., Dec.

[b] Does not apply to control farms.

TABLE 11-25. SEASONAL ON-AND OFF-FARM WORK[a] (AVERAGE NO. OF HOURS PER WEEK) PROFILES OF CONTROL AND SLUDGE POPULATIONS IN CLARK COUNTY

Type of Time	Sludge				
	All Seasons	Winter	Spring	Summer	Autumn
Total Hours per Week	168.0	168.0	168.0	168.0	168.0
Field Work	9.3	2.5	13.8	17.9	13.3
On Sludge Land[b]	0.6	0.2	0.6	0.7	0.9
Livestock Work	7.9	8.6	8.7	8.2	5.9
Off-Farm Time	39.1	38.5	34.5	39.4	44.9
School	2.9	3.1	3.3	0.6	4.7
Job	9.8	9.1	8.8	9.5	12.0
Other	26.4	26.3	22.4	29.3	28.2
	Control				
Total Hours per Week	168.0	168.0	168.0	168.0	168.0
Field Work	15.2	4.0	18.2	14.2	24.7
On Sludge Land[b]	-----	-----	-----	-----	-----
Livestock Work	5.8	6.9	6.0	4.5	5.8
Off-Farm Time	38.5	39.2	37.6	37.0	39.1
School	1.6	2.0	1.3	0.5	2.4
Job	5.0	5.0	5.5	4.5	4.9
Other	31.9	32.2	30.8	33.0	31.8

[a] Number of hours per week was calculated by averaging the monthly responses for each season over the three year period, beginning the first monthly interview following the initial sludge application and the corresponding monthly interview for controls. Winter = Jan., Feb., March; Spring = April, May, June; Summer = July, Aug., Sept.; Autumn = Oct., Nov., Dec.

[b] Does not apply to control farms.

TABLE 11-26. COMPARISON OF AMOUNT OF HOME PRODUCED FOOD CONSUMED BY SLUDGE AND CONTROL POPULATIONS IN ALL COUNTIES BY SEASON[a]

		Percentage of Total Consumption That Was Home Raised				
Food Item	Group	All Seasons	Winter	Spring	Summer	Autumn
Meat	Sludge	35.6	37.6	32.9	35.1	37.0
	Control	27.9	27.8	28.4	26.4	28.8
Fruits or Vegetables						
	Sludge	34.9	34.8	29.5	36.3	39.1
	Control	33.3	32.6	29.7	35.2	35.5

[a] Winter = Jan., Feb., March; Spring = April, May, June; Summer = July, Aug., Sept.; Autumn = Oct., Nov., Dec.

TABLE 11-27. COMPARISON OF AMOUNT OF HOME PRODUCED FOOD CONSUMED BY SLUDGE AND CONTROL POPULATIONS IN MEDINA COUNTY BY SEASON[a]

		Percentage of Total Consumption That Was Home Raised				
Food Item	Group	All Seasons	Winter	Spring	Summer	Autumn
Meat	Sludge	31.7	32.8	32.3	35.8	29.9
	Control	29.6	26.5	30.0	30.3	31.4
Fruits or Vegetables						
	Sludge	43.5	42.6	38.8	43.6	49.1
	Control	42.9	42.5	36.0	42.5	50.3

[a] Winter = Jan., Feb., March; Spring = April, May, June; Summer = July, Aug., Sept.; Autumn = Oct., Nov., Dec.

TABLE 11-28. COMPARISON OF AMOUNT OF HOME PRODUCED FOOD CONSUMED BY SLUDGE AND CONTROL POPULATIONS IN FRANKLIN-PICKAWAY COUNTIES BY SEASON[a]

Food Item	Group	Percentage of Total Consumption That Was Home Raised				
		All Seasons	Winter	Spring	Summer	Autumn
Meat	Sludge	38.2	39.6	35.4	38.3	39.6
	Control	23.4	24.9	23.3	21.2	24.3
Fruits or Vegetables						
	Sludge	36.4	37.7	31.6	36.1	40.4
	Control	32.2	31.8	30.5	32.9	33.4

[a] Winter = Jan., Feb., March; Spring = April, May, June; Summer = July, Aug., Sept.; Autumn = Oct., Nov., Dec.

TABLE 11-29. COMPARISON OF AMOUNT OF HOME PRODUCED FOOD CONSUMED BY SLUDGE AND CONTROL POPULATIONS IN CLARK COUNTY BY SEASON[a]

Food Item	Group	Percentage of Total Consumption That Was Home Raised				
		All Seasons	Winter	Spring	Summer	Autumn
Meat						
	Sludge	31.9	36.5	26.6	28.5	35.9
	Control	38.4	36.5	40.9	37.3	38.9
Fruits or Vegetables						
	Sludge	23.0	20.6	15.9	30.0	26.0
	Control	27.3	25.5	22.1	34.7	27.2

[a] Winter = Jan., Feb., March; Spring = April, May, June; Summer = July, Aug., Sept.; Autumn = Oct., Nov., Dec.

TABLE 11-30. SUMMARY OF TUBERCULIN TESTS FOR SLUDGE AND CONTROL GROUPS BY SLUDGE APPLICATION PERIOD, ALL COUNTIES

Group	Pre-Sludge Baseline	Years Post Sludge[a] 1	2	3
Sludge				
No. Tine Tests	153	148	131	80
No. Tine Significant Reactions[b]	7	1	2	0
No. Mantoux Test	6	3	4[d]	1
No. Mantoux Significant Reactions[c]	2	0	0	0
Control				
No. Tine Tests	119	114	97	57
No. Tine Significant Reactions	5[d]	0	0	0
No. Mantoux Test	2	2	2	1[e]
No. Mantoux Significant Reactions[c]	0	0	0	0

[a] Post sludge period for controls represents the period beginning with the date of interview closest to the date of first sludge application for the corresponding sludge farm.

[b] Tine test significant reaction is defined as any response of induration 5 mm or more. Prior to September 1979 those reporting a reaction to tine test were referred to their family physician for further evaluation. After September 1979 individuals who reported a significant reaction to tine test were given a Mantoux test. In subsequent years, only Mantoux test was given to these individuals.

[c] Mantoux significant reaction is an induration of 10 mm or more at the site of intermediate strength PPD injection.

[d] Of the five tine significant reactions, 3 were before 9/79 which were not Mantoux tested nor tine tested subsequently. The other two were only Mantoux tested in subsequent years.

[e] Only one participant who was Mantoux tested, was in the project for 3 years post-sludge.

TABLE 11-31. SUMMARY OF TUBERCULIN TESTS FOR SLUDGE AND CONTROL GROUPS BY SLUDGE APPLICATION PERIOD, MEDINA COUNTY

Group	Pre-Sludge Baseline	Years Post Sludge[a]		
		1	2	3
Sludge				
No. Tine Tests	29	28	17	15
No. Tine Significant Reactions[b]	2	1[e]	0	0
No. Mantoux Test	1	0	NA[f]	NA
No. Mantoux Significant Reactions[c]	1[d]	0	NA	NA
Control				
No. Tine Tests	24	25	17	19
No. Significant Reactions	0	0	0	0
No. Mantoux Tests	NA	NA	NA	NA
No. Mantoux Significant Reactions[c]	NA	NA	NA	NA

[a] Post sludge period for controls represents the period beginning with the date of interview closest to the date of first sludge application for the corresponding sludge farm.

[b] Tine test significant reaction is defined as any response of induration 5 mm or more. Prior to September 1979 those reporting a reaction to tine test were referred to their family physician for further evaluation. After September 1979 individuals who reported a significant reaction to tine test were given a Mantoux test.

[c] Mantoux significant reaction is an induration of 10 mm or more at the site of intermediate strength PPD injection.

[d] One participant had a significant Mantoux test reaction. This individual was negative on chest X ray and excluded from subsequent testing. The other individual was referred to his physician (before Sept. 1979) and subsequently dropped out of the project.

[e] This person was also positive on the pre-sludge tine test.

[f] NA means "Not Applicable".

TABLE 11-32. SUMMARY OF TUBERCULIN TESTS FOR SLUDGE AND CONTROL GROUPS BY SLUDGE APPLICATION PERIOD, FRANKLIN AND PICKAWAY COUNTIES

Group	Pre-Sludge Baseline	Years Post Sludge[a]		
		1	2	3
Sludge				
No. Tine Tests	94	89	96	48
No. Tine Significant Reactions[b]	2	0	2[d]	0
No. Mantoux Test	2	2	2[d]	NA[e]
No. Mantoux Significant Reactions[c]	0	0	0	NA
Control				
No. Tine Tests	69	66	68	27
No. Tine Significant Reactions	5[f]	0	0	0
No. Mantoux Test	2	2	2	1[g]
No. Mantoux Significant Reactions[c]	0	0	0	0

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[a] Post sludge period for controls represents the period beginning with the date of interview closest to the date of first sludge application for the corresponding sludge farm.

[b] Tine test significant reaction is defined as any response of induration 5 mm or more. Prior to September 1979 those reporting a reaction to tine test were referred to their family physician for further evaluation. After September 1979 individuals who reported a significant reaction to tine test were given a Mantoux test. In subsequent years, only Mantoux test was given to these individuals.

[c] Mantoux significant reaction is an induration of 10 mm or more at the site of intermediate strength PPD injection.

[d] Represents one individual who had been Mantoux tested in the previous year and one of the two individuals reporting new tine significant reaction during the second year. One of the individuals Mantoux tested in the previous year expired. One of the individuals with new tine significant reaction was not given the Mantoux test at the request of the physician.

[e] NA means "Not applicable".

[f] Of the 5 tine significant reactions, 3 were before 9/79 which were not Mantoux tested nor tine tested subsequently. The other two were not tine tested in subsequent years.

[g] Only one participant who was Mantoux tested, was in the project for 3 years post-sludge.

TABLE 11-33. SUMMARY OF TUBERCULIN TESTS FOR SLUDGE AND CONTROL GROUPS BY SLUDGE APPLICATION PERIOD, CLARK COUNTY

Group	Pre-Sludge Baseline	Years Post Sludge[a] 1	2	3
Sludge				
No. Tine Tests	30	31	18	17
No. Significant Reactions[b]	3	0	0	0
No. Mantoux Test	3[d]	1	2	1
No. Mantoux Significant Reactions[c]	1	0	0	0
Control				
No. Tine Tests	26	23	12	11
No. Tine Significant Reactions	0	0	0	0
No. Mantoux Test	NA[e]	NA	NA	NA
No. Mantoux Significant Reactions[c]	NA	NA	NA	NA

[a] Post sludge period for controls represents the period beginning with the date of interview closest to the date of first sludge application for the corresponding sludge farm.

[b] Tine test significant reaction is defined as any response of induration 5 mm or more. Prior to September 1979 those reporting a reaction to tine test were referred to their family physician for further evaluation. After September 1979 individuals who reported a significant reaction to tine test were given a Mantoux test.

[c] Mantoux significant reaction is an induration of 10 mm or more at the site of intermediate strength PPD injection.

[d] Out of three Mantoux tests done one was significant and excluded from subsequent testing. A chest X ray was done on the person with the significant Mantoux reaction and reported negative. One participant was not tested at this time but tested the following year.

[e] NA means "Not applicable"

TABLE 11-34. HUMAN ILLNESS RATES ON SLUDGE AND CONTROL FARMS, BY COUNTY

County	No. of Persons at Risk	No. of Persons Ill	No. of Reported Illnesses	Illness Rate Per 100 Persons at Risk	Illness Rate Per 100 Person-Years at Risk[a]
All Counties					
Sludge	168	161	874	520	261
Control	130	126	831	639	325
Medina					
Sludge	31	30	135	435	207
Control	26	25	228	877	378
Franklin and Pickaway					
Sludge	101	98	525	520	246
Control	76	76	481	633	319
Clark					
Sludge	36	33	214	594	384
Control	28	25	122	436	274

[a] Person-years at risk values were calculated by adding participation periods for all individuals at risk during the post sludge period. For controls, participation started on the date of interview following the date of sludge application on its corresponding sludge farm.

TABLE 11-35. HUMAN ILLNESS RATES FOR SELECTED SYMPTOMS IN SLUDGE AND CONTROL FARMS, ALL COUNTIES

Symptoms	Number of Reported Episodes	Rate per 100 Person Years at Risk	Persons Affected	Percentage of Population with Symptoms
All Reported Illnesses				
Sludge	874	261.4	161	96
Control	831	325.1	126	97
Fever				
Sludge	208	62.2	95	57
Control	169	66.1	75	58
Headache				
Sludge	203	60.7	100	60
Control	184	72.0	76	58
GMAP[a]				
Sludge	162	48.4	93	55
Control	134	52.4	63	48
Nausea				
Sludge	160	47.8	84	50
Control	136	53.2	65	50
Diarrhea				
Sludge	122	36.5	72	43
Control	137	53.6	69	53
Runny Nose				
Sludge	339	101.4	136	81
Control	336	131.5	103	82
Cough				
Sludge	293	87.6	127	76
Control	299	117.0	102	78
Sore Throat				
Sludge	257	76.9	113	67
Control	249	97.4	83	64
Nasal Congestion				
Sludge	314	93.9	137	82
Control	314	122.8	103	79
Chest Congestion				
Sludge	136	40.7	87	52
Control	125	48.9	69	53
Hoarseness				
Sludge	87	26.0	62	37
Control	86	33.6	53	41
Other				
Sludge	274	81.9	113	67
Control	267	104.5	95	73

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[a] GMAP stands for generalized muscular aches and pains.

TABLE 11-36. HUMAN ILLNESS RATES FOR SELECTED SYMPTOMS IN SLUDGE AND CONTROL FARMS, MEDINA COUNTY

Symptoms	Number of Reported Episodes	Rate per 100 Person Years at Risk	Persons Affected	Percentage of Population with Symptoms
All Reported Illnesses				
Sludge	135	206.7	30	97
Control	228	377.5	25	96
Fever				
Sludge	42	64.3	18	58
Control	65	107.6	16	62
Headache				
Sludge	37	56.7	21	68
Control	69	114.2	19	73
GMAP[a]				
Sludge	25	38.3	18	58
Control	52	86.1	17	65
Nausea				
Sludge	27	41.3	17	55
Control	50	82.8	14	54
Diarrhea				
Sludge	22	33.7	14	45
Control	38	62.9	14	54
Runny Nose				
Sludge	54	82.7	25	81
Control	91	150.7	18	69
Cough				
Sludge	51	78.1	23	74
Control	106	175.5	20	77
Sore Throat				
Sludge	32	49.0	20	65
Control	88	145.7	18	69
Nasal Congestion				
Sludge	50	76.6	24	77
Control	102	168.9	22	85
Chest Congestion				
Sludge	26	39.8	18	58
Control	41	67.9	16	62
Hoarseness				
Sludge	21	32.2	15	48
Control	25	41.4	12	46
Other				
Sludge	38	58.2	21	68
Control	49	81.1	15	58

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[a] GMAP stands for generalized muscular aches and pains.

TABLE 11-37. HUMAN ILLNESS RATES FOR SELECTED SYMPTOMS IN SLUDGE AND CONTROL FARMS, FRANKLIN AND PICKAWAY COUNTIES

Symptoms	Number of Reported Episodes	Rate per 100 Person Years at Risk	Persons Affected	Percentage of Population with Symptoms
All Reported Illnesses				
Sludge	525	246.0	98	97
Control	481	319.4	76	100
Fever				
Sludge	112	52.5	56	55
Control	79	52.5	44	58
Headache				
Sludge	108	50.6	55	54
Control	93	61.8	46	61
GMAP[a]				
Sludge	88	41.2	53	52
Control	63	41.8	35	46
Nausea				
Sludge	92	43.1	45	45
Control	71	47.1	38	50
Diarrhea				
Sludge	64	30.0	40	40
Control	79	52.5	42	55
Runny Nose				
Sludge	205	96.1	81	80
Control	198	131.5	66	87
Cough				
Sludge	177	82.9	77	76
Control	155	102.9	62	82
Sore Throat				
Sludge	150	70.3	65	64
Control	137	91.0	52	68
Nasal Congestion				
Sludge	190	89.0	84	83
Control	169	112.2	62	82
Chest Congestion				
Sludge	76	35.6	50	50
Control	73	48.5	44	58
Hoarseness				
Sludge	42	19.7	32	32
Control	49	32.5	30	39
Other				
Sludge	174	81.5	71	70
Control	171	113.5	63	83

[a] GMAP stands for generalized muscular aches and pains.

TABLE 11-38. HUMAN ILLNESS RATES FOR SELECTED SYMPTOMS IN SLUDGE AND CONTROL FARMS, CLARK COUNTY

Symptoms	Number of Reported Episodes	Rate per 100 Person Years at Risk	Persons Affected	Percentage of Population with Symptoms
All Reported Illnesses				
Sludge	214	384.2	33	92
Control	122	273.5	25	89
Fever				
Sludge	54	96.9	21	58
Control	25	56.1	15	54
Headache				
Sludge	58	104.1	24	67
Control	22	49.3	11	39
GMAP[a]				
Sludge	49	88.0	22	61
Control	19	42.6	11	39
Nausea				
Sludge	41	73.6	22	61
Control	15	33.6	13	46
Diarrhea				
Sludge	36	64.6	18	50
Control	20	44.8	13	46
Runny Nose				
Sludge	80	143.6	30	83
Control	47	105.4	19	68
Cough				
Sludge	66	118.5	27	75
Control	38	85.2	20	71
Sore Throat				
Sludge	75	134.6	28	78
Control	24	53.8	13	46
Nasal Congestion				
Sludge	74	132.9	29	81
Control	43	96.4	19	68
Chest Congestion				
Sludge	34	61.0	19	53
Control	11	24.7	9	32
Hoarseness				
Sludge	24	43.1	15	42
Control	12	26.9	11	39
Other				
Sludge	62	111.3	21	58
Control	47	105.4	17	61

[a] GMAP stands for generalized muscular aches and pains.

TABLE 11-39. MATCHED PAIR LINEAR LOGISTIC REGRESSION ANALYSIS OF SINGLE AND COMBINED SYMPTOMS BETWEEN SLUDGE AND CONTROL FARMS, FIRST SLUDGE APPLICATION

Symptoms	Matched Pair[a]		Matched Pair[a] Adjusting for Additional Confounders[b]	
	Odds Ratio	P Value	Odds Ratio	P Value
Single:				
Fever	1.00	0.99	0.85	0.83
Headache	0.76	0.46	1.50	0.50
GMAP[c]	0.86	0.70	0.34	0.15
Nausea	0.92	0.84	1.12	0.88
Diarrhea	0.91	0.83	0.26	0.10
Runny Nose	1.20	0.55	0.90	0.79
Sore Throat	1.11	0.75	0.83	0.66
Nasal Congestion	1.00	0.99	2.00	0.46
Hoarseness	0.75	0.51	0.45	0.34
Chest Congestion	1.39	0.29	1.35	0.52
Cough	1.05	0.88	0.96	0.92
Any of Above	1.16	0.53	1.05	0.88
Combined:				
General	1.09	0.76	0.82	0.64
Digestive	0.76	0.47	0.38	0.09
Upper Respiratory	1.32	0.33	1.05	0.90
Lower Respiratory	1.10	0.76	0.99	0.99

[a] Matched for county and period of observation.

[b] Age (5 categories) and weeks of observation.

[c] Generalized muscular aches and pains.

TABLE 11-40. MATCHED PAIR LINEAR LOGISTIC REGRESSION ANALYSIS OF SINGLE AND COMBINED SYMPTOMS BETWEEN SLUDGE AND CONTROL FARMS, SECOND SLUDGE APPLICATION

Symptoms	Matched Pair[a]		Matched Pair[a] Adjusting for Additional Confounders[b]	
	Odds Ratio	P Value	Odds Ratio	P Value
Single:				
Fever	1.50	0.53	1.39	0.66
Headache	1.80	0.29	1.37	0.71
GMAP[c]	2.00	0.33	d	0.83
Nausea	2.25	0.18	6.04	0.16
Diarrhea	2.00	0.33	4.42	0.43
Runny Nose	1.10	0.83	0.69	0.62
Sore Throat	1.00	1.00	1.05	0.26
Nasal Congestion	1.60	0.41	12.11	0.94
Hoarseness	0.83	0.76	0.98	0.89
Chest Congestion	2.14	0.10	1.98	0.75
Cough	1.08	0.84	0.23	0.28
Any of Above	1.37	0.33	1.13	0.80
Combined:				
General	1.75	0.21	1.55	0.56
Digestive	1.80	0.29	2.69	0.41
Upper Respiratory	0.81	0.59	1.21	0.72
Lower Respiratory	1.42	0.36	0.72	0.60

[a] Matched for county and period of observation.

[b] Age (5 categories) and weeks of observation.

[c] Generalized muscular aches and pains.

[d] Value not reported due to high standard error.

TABLE 11-41. COMPARISON OF THE FREQUENCY OF REPORTED NEW ILLNESSES AND SELECTED SYMPTOMS FOR PERSONS IN SLUDGE AND CONTROL GROUPS FOR 7 WEEK PRE-SLUDGE (PRE-S) APPLICATION AND 7 WEEK POST-SLUDGE (POST-S) APPLICATION PERIODS FOLLOWING EACH SLUDGE APPLICATION, ALL COUNTIES

Symptom and Study Group	Pre-S	Applications[a]					
		1st Post-S	2nd Post-S	3rd Post-S	4th Post-S	5th Post-S	6th Post-S
All Illnesses							
Sludge	30 (19)	45 (19)	36 (21)	17 (18)	7 (13)	4 (25)	2 (20)
Control	38 (31)	56 (29)	29 (22)	19 (28)	11 (25)	5 (42)	1 (25)
Fever							
Sludge	7 (4)	11 (5)	8 (5)	1 (1)	2 (4)	0 (0)	0 (0)
Control	9 (7)	11 (6)	5 (4)	2 (3)	4 (9)	0 (0)	0 (0)
Headache							
Sludge	15 (10)	10 (4)	7 (4)	2 (2)	0 (0)	0 (0)	0 (0)
Control	8 (7)	13 (7)	3 (2)	4 (6)	3 (7)	0 (0)	0 (0)
GMAP[b]							
Sludge	13 (8)	15 (6)	4 (2)	0 (0)	1 (2)	0 (0)	0 (0)
Control	2 (2)	15 (8)	1 (1)	2 (3)	1 (2)	0 (0)	0 (0)
Nausea							
Sludge	6 (4)	11 (5)	2 (1)	1 (1)	0 (0)	0 (0)	0 (0)
Control	9 (7)	9 (5)	3 (2)	3 (4)	4 (9)	0 (0)	0 (0)
Diarrhea							
Sludge	5 (3)	9 (4)	7 (4)	0 (0)	0 (0)	0 (0)	0 (0)
Control	4 (3)	13 (7)	2 (2)	1 (1)	2 (5)	1 (8)	0 (0)
Runny Nose							
Sludge	9 (6)	17 (7)	14 (8)	2 (2)	2 (4)	2 (12)	2 (20)
Control	20 (16)	26 (13)	8 (6)	7 (10)	2 (5)	0 (0)	0 (0)
Cough							
Sludge	15 (10)	17 (7)	12 (7)	3 (3)	1 (4)	2 (12)	0 (10)
Control	22 (18)	27 (14)	9 (7)	9 (13)	2 (5)	1 (8)	0 (0)
Sore Throat							
Sludge	11 (7)	13 (6)	12 (7)	1 (1)	2 (4)	2 (12)	1 (10)
Control	16 (13)	19 (10)	12 (9)	7 (10)	2 (5)	0 (0)	0 (0)
Nasal congestion							
Sludge	12 (8)	20 (8)	13 (8)	5 (5)	2 (4)	1 (6)	1 (10)
Control	19 (15)	29 (15)	5 (4)	10 (15)	2 (5)	0 (0)	1 (25)
Chest congestion							
Sludge	5 (3)	5 (2)	3 (2)	0 (0)	0 (0)	1 (6)	1 (10)
Control	10 (8)	18 (9)	3 (2)	5 (7)	0 (0)	0 (0)	0 (0)
Hoarseness							
Sludge	4 (3)	5 (2)	3 (2)	0 (0)	0 (0)	0 (0)	1 (10)
Control	8 (7)	14 (7)	4 (3)	3 (4)	0 (0)	0 (0)	0 (0)
Other							
Sludge	14 (9)	14 (6)	16 (9)	13 (14)	2 (4)	2 (12)	0 (0)
Control	12 (10)	17 (9)	13 (10)	9 (13)	4 (9)	3 (25)	0 (0)

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[a] Number reported (percentage of responses with illness).

[b] GMAP stands for generalized muscular aches and pains.

TABLE 11-42. COMPARISON OF THE FREQUENCY OF REPORTED NEW ILLNESSES AND SELECTED SYMPTOMS FOR PERSONS IN SLUDGE AND CONTROL GROUPS FOR 7 WEEK PRE-SLUDGE (PRE-S) APPLICATION AND 7 WEEK POST-SLUDGE (POST-S) APPLICATION PERIODS FOLLOWING EACH SLUDGE APPLICATION, MEDINA COUNTY

Symptom and Study Group	Pre-S	Applications[a]				
		1st Post-S	2nd Post-S	3rd Post-S	4th Post-S	5th Post-S
All Illnesses						
Sludge	8 (17)	12 (19)	6 (12)	0 (0)	3 (12)	1 (17)
Control	14 (31)	15 (29)	12 (27)	10 (39)	9 (28)	3 (38)
Fever						
Sludge	1 (2)	1 (2)	2 (4)	0 (0)	1 (4)	0 (0)
Control	4 (9)	5 (10)	4 (9)	1 (4)	4 (14)	0 (0)
Headache						
Sludge	2 (4)	3 (5)	1 (2)	0 (0)	0 (0)	0 (0)
Control	2 (4)	5 (10)	0 (0)	4 (15)	3 (11)	0 (0)
GMAP[b]						
Sludge	3 (6)	3 (5)	1 (2)	0 (0)	0 (0)	0 (0)
Control	2 (4)	6 (12)	1 (2)	0 (0)	1 (4)	0 (0)
Nausea						
Sludge	2 (4)	2 (3)	1 (2)	0 (0)	0 (0)	0 (0)
Control	5 (11)	4 (8)	0 (0)	1 (4)	4 (14)	0 (0)
Diarrhea						
Sludge	1 (2)	3 (5)	0 (0)	0 (0)	0 (0)	0 (0)
Control	4 (9)	6 (12)	1 (2)	1 (4)	2 (7)	0 (0)
Runny Nose						
Sludge	3 (6)	3 (5)	1 (2)	0 (0)	2 (8)	0 (0)
Control	7 (16)	10 (19)	4 (9)	4 (15)	2 (7)	0 (0)
Cough						
Sludge	3 (6)	4 (6)	2 (4)	0 (0)	1 (4)	0 (0)
Control	11 (24)	9 (17)	5 (11)	6 (23)	2 (7)	0 (0)
Sore Throat						
Sludge	3 (6)	5 (8)	1 (2)	0 (0)	1 (4)	0 (0)
Control	6 (13)	6 (12)	5 (11)	4 (15)	2 (7)	0 (0)
Nasal Congestion						
Sludge	3 (6)	4 (6)	1 (2)	0 (0)	2 (8)	0 (0)
Control	9 (20)	13 (25)	2 (4)	6 (23)	2 (7)	0 (0)
Chest Congestion						
Sludge	0 (0)	1 (2)	2 (4)	0 (0)	0 (0)	0 (0)
Control	6 (13)	7 (13)	1 (2)	4 (15)	0 (0)	0 (0)
Hoarseness						
Sludge	1 (2)	3 (5)	2 (4)	0 (0)	0 (0)	0 (0)
Control	4 (9)	7 (13)	1 (2)	3 (12)	0 (0)	0 (0)
Other						
Sludge	4 (9)	3 (5)	1 (2)	0 (0)	0 (0)	1 (17)
Control	2 (4)	2 (4)	3 (7)	3 (12)	2 (7)	3 (38)

[a] Number Reported (percentage of responses with illness)

[b] GMAP stands for generalized muscular aches and pains.

TABLE 11-43. COMPARISON OF THE FREQUENCY OF REPORTED NEW ILLNESSES AND SELECTED SYMPTOMS FOR PERSONS IN SLUDGE AND CONTROL GROUPS FOR 7 WEEK PRE-SLUDGE (PRE-S) APPLICATION AND 7 WEEK POST-SLUDGE (POST-S) APPLICATION PERIODS FOLLOWING EACH SLUDGE APPLICATION. FRANKLIN AND PICKAWAY COUNTIES

Symptom and Study Group	Pre-S	Applications[a]					
		1st Post-S	2nd Post-S	3rd Post-S	4th Post-S	5th Post-S	6th Post-S
All Illnesses							
Sludge	19 (19)	31 (19)	27 (25)	15 (25)	3 (11)	3 (30)	2 (20)
Control	19 (28)	38 (30)	16 (21)	7 (19)	1 (10)	2 (50)	1 (25)
Fever							
Sludge	5 (5)	10 (6)	6 (6)	1 (2)	1 (4)	1 (10)	0 (0)
Control	5 (7)	6 (5)	1 (1)	1 (3)	0 (0)	0 (0)	0 (0)
Headache							
Sludge	11 (11)	6 (4)	4 (4)	2 (3)	0 (0)	0 (0)	0 (0)
Control	5 (7)	8 (6)	3 (4)	0 (0)	0 (0)	0 (0)	0 (0)
GMAP[b]							
Sludge	8 (8)	11 (7)	2 (2)	0 (0)	1 (4)	1 (10)	0 (0)
Control	0 (0)	9 (7)	0 (0)	2 (6)	0 (0)	0 (0)	0 (0)
Nausea							
Sludge	3 (3)	9 (6)	0 (0)	1 (2)	0 (0)	0 (0)	0 (0)
Control	4 (6)	5 (4)	3 (4)	1 (3)	0 (0)	0 (0)	0 (0)
Diarrhea							
Sludge	3 (3)	5 (3)	5 (5)	0 (0)	0 (0)	0 (0)	0 (0)
Control	0 (0)	5 (4)	1 (1)	0 (0)	0 (0)	1 (25)	0 (0)
Runny Nose							
Sludge	6 (6)	14 (9)	11 (10)	2 (3)	0 (0)	2 (20)	2 (20)
Control	10 (14)	16 (12)	4 (5)	2 (6)	0 (0)	0 (0)	0 (0)
Cough							
Sludge	10 (10)	12 (8)	8 (7)	2 (3)	0 (0)	2 (20)	1 (10)
Control	9 (13)	17 (13)	4 (5)	2 (6)	0 (0)	1 (25)	0 (0)
Sore Throat							
Sludge	8 (8)	8 (5)	8 (7)	1 (2)	1 (4)	2 (20)	1 (10)
Control	9 (13)	13 (10)	6 (8)	2 (6)	0 (0)	0 (0)	0 (0)
Nasal Congestion							
Sludge	9 (9)	16 (10)	11 (10)	5 (8)	0 (0)	1 (10)	1 (10)
Control	7 (10)	16 (12)	3 (4)	3 (8)	0 (0)	0 (0)	1 (25)
Chest Congestion							
Sludge	3 (3)	3 (2)	1 (1)	0 (0)	0 (0)	1 (10)	1 (10)
Control	4 (6)	10 (8)	2 (3)	1 (3)	0 (0)	0 (0)	0 (0)
Hoarseness							
Sludge	3 (3)	1 (1)	1 (1)	0 (0)	0 (0)	0 (0)	1 (10)
Control	4 (6)	6 (5)	3 (4)	0 (0)	0 (0)	0 (0)	0 (0)
Other							
Sludge	9 (9)	9 (6)	14 (13)	11 (18)	1 (4)	1 (10)	0 (0)
Control	7 (10)	15 (12)	10 (13)	4 (11)	1 (10)	0 (0)	0 (0)

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[a] Number reported (percentage of responses with illness).

[b] GMAP stands for generalized muscular aches and pains.

TABLE 11-44. COMPARISON OF THE FREQUENCY OF REPORTED NEW ILLNESSES AND SELECTED SYMPTOMS FOR PERSONS IN SLUDGE AND CONTROL GROUPS FOR 7 WEEK PRE-SLUDGE (PRE-S) APPLICATION AND 7 WEEK POST-SLUDGE (POST-S) APPLICATION PERIODS FOLLOWING EACH SLUDGE APPLICATION, CLARK COUNTY

Symptom and Study Group	Pre-S	Applications[a]				
		1st Post-S	2nd Post-S	3rd Post-S	4th Post-S	5th Post-S
All Illnesses						
Sludge	3 (27)	2 (14)	3 (25)	2 (50)	1 (50)	0 (0)
Control	5 (56)	3 (21)	1 (7)	2 (33)	1 (50)	0 (0)
Fever						
Sludge	1 (9)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Control	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Headache						
Sludge	2 (18)	1 (7)	2 (17)	0 (0)	0 (0)	0 (0)
Control	1 (11)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
GMAP[b]						
Sludge	2 (18)	1 (7)	1 (12)	0 (0)	0 (0)	0 (0)
Control	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Nausea						
Sludge	1 (9)	0 (0)	1 (12)	0 (0)	0 (0)	0 (0)
Control	0 (0)	0 (0)	0 (0)	1 (17)	0 (0)	0 (0)
Diarrhea						
Sludge	1 (9)	1 (7)	2 (25)	0 (0)	0 (0)	0 (0)
Control	0 (0)	2 (14)	0 (0)	0 (0)	0 (0)	0 (0)
Runny Nose						
Sludge	0 (0)	0 (0)	2 (25)	0 (0)	0 (0)	0 (0)
Control	3 (33)	0 (0)	0 (0)	1 (17)	0 (0)	0 (0)
Cough						
Sludge	2 (18)	1 (7)	2 (25)	1 (25)	0 (0)	0 (0)
Control	2 (22)	1 (7)	0 (0)	1 (17)	0 (0)	0 (0)
Sore Throat						
Sludge	0 (0)	0 (0)	3 (38)	0 (0)	0 (0)	0 (0)
Control	1 (11)	0 (0)	1 (7)	1 (17)	0 (0)	0 (0)
Nasal Congestion						
Sludge	0 (0)	0 (0)	1 (12)	0 (0)	0 (0)	0 (0)
Control	3 (33)	0 (0)	0 (0)	1 (17)	0 (0)	0 (0)
Chest Congestion						
Sludge	2 (18)	1 (7)	0 (0)	0 (0)	0 (0)	0 (0)
Control	0 (0)	1 (7)	0 (0)	0 (0)	0 (0)	0 (0)
Hoarseness						
Sludge	0 (0)	1 (7)	0 (0)	0 (0)	0 (0)	0 (0)
Control	0 (0)	1 (7)	0 (0)	0 (0)	0 (0)	0 (0)
Other						
Sludge	1 (9)	2 (14)	1 (12)	2 (50)	1 (50)	0 (0)
Control	3 (33)	0 (0)	0 (0)	2 (33)	1 (50)	0 (0)

[a] Number reported (percentage of responses with illness).

[b] GMAP stands for generalized muscular aches and pains.

TABLE 11-45. HUMAN ILLNESS RATES AND NUMBER OF HOURS OF SLUDGE EXPOSURE PER WEEK

Mean No. Hrs. of Sludge Exposure per Wk.	Number of Participants	Number of Illnesses	Illness Rate per 100 Person-years at Risk
TOTAL	168	874	261.4
0	30	172	314.5
>0 - 10 min.	34	170	247.3
>10 min. - 45 min.	36	176	275.8
>45 min. - 1 1/2 hr.	32	166	257.1
>1 1/2 hr.	36	190	230.2

TABLE 11-46. SEROCONVERSIONS[a] TO COXSACKIE A (CA), COXSACKIE B (CB) AND ECHO (EC) VIRUSES, ASSOCIATED SYMPTOMS AND PHYSICIAN VISITS AMONG SLUDGE AND CONTROL FARM RESIDENTS

Virus	No. of Seroconversions on Sludge Farms				No. of Seroconversions on Control Farms			
	Total	With Respiratory Symptoms	With Digestive Symptoms	Visited Physician	Total	With Respiratory Symptoms	With Digestive Symptoms	Visited Physician
All	15	10	3	5	14	6	6	0
CA 3	2	2	0	2	1	0	0	0
CA 7	1	1	1	0	1	0	0	0
CB 2	1	1	0	1	1	1	1	0
CB 3	0	0	0	0	0	0	0	0
CB 4	1	1	1	0	2	1	0	0
EC 3	1	0	0	0	0	0	0	0
EC 6	1	0	1	0	0	0	0	0
EC 7	0	0	0	0	1	0	1	0
EC 9	1	1	0	1	2	1	0	0
EC 11	0	0	0	0	1	0	1	0
EC 20	1	0	0	0	1	1	0	0
EC 21	1	0	0	0	1	0	0	0
EC 25	5	4	0	1	3	2	3	0
EC 26	0	0	0	0	1	0	0	0

[a] Persons with a fourfold rise in antibody titer between sequential serum samples collected during the sludge application period.

TABLE 11-47. SEVERITY OF COXSACKIE A (CA), COXSACKIE B (CB) AND ECHO (EC) VIRUS INFECTIONS[a] AMONG SLUDGE AND CONTROL FARM RESIDENTS DURING THE SLUDGE APPLICATION PERIOD

Virus	Sludge Farm Residents Illness Status				Control Farm Residents Illness Status			
	Not Ill	Ill, Worked	Ill, At Home	Ill, Bed	Not Ill	Ill, Worked	Ill, At Home	Ill, Bed
All	4	5	5	1	5	3	3	3
CA 3			2		1			
CA 7		1				1		
CB 2		1						
CB 3					1			1
CB 4			1					
EC 3	1							
EC 6				1				
EC 7								1
EC 9			1			1		
EC 11							1	
EC 20	1					1		
EC 21	1				1			
EC 25	1	3	1		1		2	1
EC 26					1			

[a] Infections were persons with fourfold rise in antibody titer between sequential serum samples; severity was defined as worst illness status recorded during period of fourfold rise.

TABLE 11-48. COMPARISON OF THE NUMBER OF ANIMALS AND ANIMAL UNITS AND THEIR RISK PERIODS BETWEEN SLUDGE AND CONTROL FARMS BY SPECIES AND TYPE OF OPERATIONS, ALL COUNTIES

Species and Type of Operation	Sludge No. of Units[a]	Sludge No. of Unit Years at Risk[b]	Sludge No. of Animal Years at Risk[c]	Control No. of Units[a]	Control No. of Unit Years at Risk[b]	Control No. of Animal Years at Risk[c]
ALL BOVINE	72	89.83	1492.85	79	108.77	1772.98
Beef Breeding	17	26.65	514.27	20	36.04	640.12
Calves	22	31.77	412.33	23	36.96	492.31
Yearlings	13	15.79	185.08	20	20.92	246.31
Feedlot Cattle	17	13.69	379.25	15	13.98	418.38
Dairy Cattle	3	1.92	1.92	1	0.87	0.87
ALL PORCINE	36	43.27	3397.10	42	59.88	3266.31
Breeding Pigs	9	13.27	377.56	13	22.42	481.52
Baby Pigs	11	9.85	836.90	12	16.06	1001.10
Fattening Pigs	16	20.15	2182.63	17	21.40	1783.69
ALL OVINE	17	19.50	411.71	11	18.46	630.12
Breeding Sheep	9	10.50	229.27	6	10.87	409.98
Fattening Sheep	8	9.00	182.44	5	7.60	220.13
ALL EQUINE	26	25.50	88.62	20	27.58	57.79
Breeding Horses	3	2.48	11.08	0	0.00	0.00
Foals	4	2.38	3.44	4	2.19	2.90
Yearlings	2	1.73	5.37	1	0.60	1.19
Pleasure Horses	17	18.90	68.73	15	24.79	53.69
ALL AVIAN	22	37.96	947.33	22	25.13	643.81
Chickens, Layers	10	22.33	725.75	10	14.13	443.17
Chickens, Broiler	4	3.40	99.17	4	2.54	70.19
Poultry, Misc.	8	12.23	122.40	8	8.46	130.44
Dogs (Canine)	45	78.27	165.81	40	67.37	127.00
Cats (Feline)	29	47.77	138.42	32	52.23	270.46

[a] Unit was defined as a group of animals of the same species and type of operation under the management of a single individual.

[b] Number of unit years at risk was calculated by adding of the number of weeks of data contribution by each unit for each interview and dividing by 52.

[c] Number of animal years at risk was calculated by adding the total number of weeks of data contribution by all animals within each unit at the time of each interview and dividing by 52.

TABLE 11-49. COMPARISON OF THE NUMBER OF ANIMALS AND ANIMAL UNITS AND THEIR RISK PERIODS BETWEEN SLUDGE AND CONTROL FARMS BY SPECIES AND TYPE OF OPERATIONS, MEDINA COUNTY

Species and Type of Operation	Sludge			Control		
	No. of Units[a]	No. of Unit Years at Risk[b]	No. of Animal Years at Risk[c]	No. of Units[a]	No. of Unit Years at Risk[b]	No. of Animal Years at Risk[c]
ALL BOVINE	15	13.90	105.27	15	20.31	128.19
Beef Breeding	3	2.42	43.23	3	5.87	43.60
Calves	5	4.98	26.44	5	6.58	41.96
Yearlings	4	4.52	21.81	3	5.04	27.44
Feedlot Cattle	3	1.98	13.79	3	1.96	14.33
Dairy Cattle	0	0.00	0.00	1	0.87	0.87
ALL PORCINE	3	2.63	20.96	10	16.91	438.09
Breeding Pigs	0	0.00	0.00	3	6.33	87.96
Baby Pigs	0	0.00	0.00	3	5.29	236.13
Fattening Pigs	3	2.63	20.96	4	5.29	114.00
ALL OVINE	6	5.87	42.25	2	4.52	386.10
Breeding Sheep	3	3.35	34.12	1	2.54	248.60
Fattening Sheep	3	2.52	10.13	1	1.98	137.92
ALL EQUINE	9	5.62	7.40	4	2.62	4.23
Breeding Horses	1	1.00	1.15	0	0.00	0.00
Foals	1	.08	0.08	1	0.23	0.23
Yearlings	0	0.00	0.00	0	0.00	0.00
Pleasure Horses	7	4.54	6.17	3	2.38	4.00
ALL AVIAN	6	8.58	217.17	5	7.10	278.13
Chickens, Layers	2	3.96	137.79	3	4.56	203.31
Chickens, Broiler	1	0.08	1.85	0	0.00	0.00
Poultry, Misc.	3	4.54	77.54	2	2.54	74.83
Dogs (Canine)	8	10.73	28.33	8	14.65	29.12
Cats (Feline)	9	12.83	51.98	4	7.90	55.31

[a] Unit was defined as a group of animals of the same species and type of operation under the management of a single individual.

[b] Number of unit years at risk was calculated by adding of the number of weeks of data contribution by each unit for each interview and dividing by 52.

[c] Number of animal years at risk was calculated by adding the total number of weeks of data contribution by all animals within each unit at the time of each interview and dividing by 52.

TABLE 11-50. A COMPARISON OF THE NUMBER OF ANIMALS AND ANIMAL UNITS AND THEIR RISK PERIODS BETWEEN SLUDGE AND CONTROL FARMS BY SPECIES AND TYPE OF OPERATIONS, FRANKLIN AND PICKAWAY COUNTIES

Species and Type of Operation	Sludge			Control		
	No. of Units[a]	No. of Unit Years at Risk[b]	No. of Animal Years at Risk[c]	No. of Units[a]	No. of Unit Years at Risk[b]	No. of Animal Years at Risk[c]
ALL BOVINE	46	62.15	1071.71	41	64.33	1261.79
Beef Breeding	10	20.37	406.94	12	22.62	455.21
Calves	14	22.77	319.50	12	22.00	314.40
Yearlings	7	8.60	122.40	11	12.58	169.46
Feedlot Cattle	11	8.50	220.94	6	7.13	322.71
Dairy Cattle	3	1.92	1.92	0	0.00	0.00
ALL PORCINE	20	26.08	1965.36	20	31.12	1988.71
Breeding Pigs	5	8.06	127.02	6	11.50	260.42
Baby Pigs	7	6.06	306.02	5	7.27	481.62
Fattening Pigs	8	11.96	1532.33	9	12.35	1246.73
ALL OVINE	9	11.90	287.37	5	8.19	103.42
Breeding Sheep	5	6.17	149.77	3	5.23	77.08
Fattening Sheep	4	5.73	137.60	2	2.96	26.35
ALL EQUINE	11	13.21	57.79	16	24.96	53.56
Breeding Horses	1	0.50	1.50	0	0.00	0.00
Foals	2	1.40	1.40	3	1.96	2.67
Yearlings	1	0.92	2.52	1	0.60	1.19
Pleasure Horses	7	10.38	52.37	12	22.40	49.69
ALL AVIAN	11	20.46	654.62	11	10.40	273.15
Chickens, Layers	6	13.52	537.94	5	5.71	176.88
Chickens, Broiler	2	2.50	86.31	2	1.06	61.31
Poultry, Misc.	3	4.44	30.37	4	3.63	34.96
Dogs (Canine)	27	50.35	105.38	23	42.50	79.25
Cats (Feline)	15	28.15	133.02	17	30.15	127.71

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[a] Unit was defined as a group of animals of the same species and type of operation under the management of a single individual.

[b] Number of unit years at risk was calculated by adding of the number of weeks of data contribution by each unit for each interview and dividing by 52.

[c] Number of animal years at risk was calculated by adding the total number of weeks of data contribution by all animals within each unit at the time of each interview and dividing by 52.

TABLE 11-51. A COMPARISON OF THE NUMBER OF ANIMALS AND ANIMAL UNITS AND THEIR RISK PERIODS BETWEEN SLUDGE AND CONTROL FARMS BY SPECIES AND TYPE OF OPERATIONS, CLARK COUNTY

Species and Type of Operation	Sludge			Control		
	No. of Units[a]	No. of Unit Years at Risk[b]	No. of Animal Years at Risk[c]	No. of Units[a]	No. of Unit Years at Risk[b]	No. of Animal Years at Risk[c]
ALL BOVINE	11	13.77	315.87	23	24.13	408.00
Beef Breeding	3	3.87	64.10	5	7.56	141.31
Calves	3	4.02	66.38	6	8.38	135.94
Yearlings	2	2.67	40.87	6	3.31	49.40
Feedlot Cattle	3	3.21	144.52	6	4.88	81.35
Dairy Cattle	0	0.00	0.00	0	0.00	0.00
ALL PORCINE	13	14.56	1410.77	12	11.87	839.44
Breeding Pigs	4	5.21	250.54	4	4.60	133.13
Baby Pigs	4	3.79	530.88	4	3.50	283.35
Fattening Pigs	5	5.56	629.35	4	3.77	422.96
ALL OVINE	2	1.73	80.09	4	5.75	140.17
Breeding Sheep	1	0.98	45.38	2	3.10	84.31
Fattening Sheep	1	0.75	34.71	2	2.65	55.87
ALL EQUINE	4	6.67	23.42	0	0.00	0.00
Breeding Horses	1	0.98	8.42	0	0.00	0.00
Foals	1	0.90	1.96	0	0.00	0.00
Yearlings	1	0.81	2.85	0	0.00	0.00
Pleasure Horses	1	3.98	10.19	0	0.00	0.00
ALL AVIAN	5	8.92	75.54	6	7.63	92.52
Chickens, Layers	2	4.85	50.02	2	3.87	62.98
Chickens, Broiler	1	0.83	11.02	2	1.48	8.88
Poultry, Misc.	2	3.25	14.50	2	2.29	20.65
Dogs (Canine)	10	17.19	31.71	9	10.21	18.63
Cats (Feline)	5	6.79	53.42	11	14.17	87.44

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[a] Unit was defined as a group of animals of the same species and type of operation under the management of a single individual.

[b] Number of unit years at risk was calculated by adding of the number of weeks of data contribution by each unit for each interview and dividing by 52.

[c] Number of animal years at risk was calculated by adding the total number of weeks of data contribution by all animals within each unit at the time of each interview and dividing by 52.

TABLE 11-52. COMPARISON OF DURATION OF TIME SPENT ON FIELD, DURATION OF SLUDGE EXPOSURE, AND CONSUMPTION OF HOME GROWN FEED IN ANIMALS LIVING ON SLUDGE AND CONTROL FARMS, ALL COUNTIES

	Sludge			Control	
Species	No. of Hours of Exposure to Sludge Treated Fields /Week	No. of Hours Spent on Fields and Pasture/ Week	Percent of Feed Home Raised	No. of Hours Spent on Fields and Pasture/ Week	Percent of Feed Home Raised
ALL BOVINE	40.2	106.3	92.4	112.0	90.1
Beef Breeding	68.0	142.3	96.1	134.1	95.0
Calves	56.6	125.7	93.3	118.3	89.6
Yearlings	8.9	79.9	88.4	97.5	88.8
Feedlot Cattle	0.1	49.3	88.4	78.9	84.3
Miscellaneous	16.9	137.0	98.6	155.1	10.7
ALL PORCINE	0.3	11.5	58.7	45.1	52.9
Breeding Pigs	2.6	28.4	48.1	65.9	63.1
Baby Pigs	0.0	12.7	53.4	20.3	63.3
Fattening Pigs	0.0	8.1	62.6	53.6	44.2
ALL OVINE	32.3	131.3	90.6	125.6	80.3
Breeding Sheep	31.6	136.5	90.3	144.2	84.0
Fattening Sheep	33.1	124.8	91.1	90.8	73.5
ALL EQUINE	0.1	118.2	75.1	145.9	73.3
Breeding Horses	0.0	86.9	87.7	0.0[a]	0.0[a]
Foals	0.0	111.2	84.1	127.9	48.9
Yearlings	0.0	116.5	68.0	168.0	82.2
Pleasure Horses	0.1	123.7	73.2	146.4	74.4
ALL AVIAN	1.6	32.1	59.8	51.3	42.0
Chickens, layers	1.7	23.0	65.1	34.7	41.2
Chickens, broiler	0.1	12.5	19.0	9.9	0.9
Miscellaneous	2.1	102.4	61.4	130.0	66.5
Dogs (Canine)	3.3	92.1	15.3	106.9	8.9
Cats (Feline)	14.2	140.1	28.4	152.7	30.0

[a] There were no breeding horses on control farms.

TABLE 11-53. COMPARISON OF DURATION OF TIME SPENT ON FIELD, DURATION OF SLUDGE EXPOSURE, AND CONSUMPTION OF HOME GROWN FEED IN ANIMALS LIVING ON SLUDGE AND CONTROL FARMS, MEDINA COUNTY

	Sludge			Control	
Species	No. of Hours of Exposure to Sludge Treated Fields /Week	No. of Hours Spent on Fields and Pasture/ Week	Percent of Feed Home Raised	No. of Hours Spent on Fields and Pasture/ Week	Percent of Feed Home Raised
ALL BOVINE	0.0	91.9	92.1	99.0	80.5
Beef Breeding	0.0	104.5	98.3	125.5	95.0
Calves	0.0	93.9	87.8	95.8	70.2
Yearlings	0.0	104.6	97.4	76.3	89.4
Feedlot Cattle	0.0	31.4	73.6	67.9	53.7
Miscellaneous	0.0[a]	0.0[a]	0.0[a]	155.1	10.7
ALL PORCINE	0.0	0.0	44.0	16.3	38.1
Breeding Pigs	0.0[a]	0.0[a]	0.0[a]	30.8	48.4
Baby Pigs	0.0[a]	0.0[a]	0.0[a]	16.9	33.7
Fattening Pigs	0.0	0.0	44.0	3.9	39.1
ALL OVINE	0.0	132.0	97.5	125.6	75.5
Breeding Sheep	0.0	124.6	97.3	145.1	75.3
Fattening Sheep	0.0	155.6	98.4	90.4	70.4
ALL EQUINE	0.1	113.0	84.3	145.8	92.3
Breeding Horses	0.0	168.0	99.0	0.0[a]	0.0[a]
Foals	0.0	98.0	0.0	0.0[a]	0.0[a]
Yearlings	0.0[a]	0.0[a]	0.0[a]	0.0[a]	0.0[a]
Pleasure Horses	0.1	104.0	81.4	154.2	97.6
ALL AVIAN	1.0	41.5	70.8	81.7	73.3
Chickens, layers	0.3	0.3	72.4	50.5	67.7
Chickens, broiler	0.0	168.6	50.0	0.0[a]	0.0[a]
Miscellaneous	2.3	111.6	68.6	166.7	88.6
Dogs (Canine)	3.4	92.9	3.8	65.9	13.3
Cats (Feline)	17.9	125.2	12.6	134.7	28.8

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[a] There were no animals in these categories.

TABLE 11-54. COMPARISON OF DURATION OF TIME SPENT ON FIELD, DURATION OF SLUDGE EXPOSURE, AND CONSUMPTION OF HOME GROWN FEED IN ANIMALS LIVING ON SLUDGE AND CONTROL FARMS, FRANKLIN-PICKAWAY COUNTIES

	Sludge			Control	
Species	No. of Hours of Exposure to Sludge Treated Fields /Week	No. of Hours Spent on Fields and Pasture/ Week	Percent of Feed Home Raised	No. of Hours Spent on Fields and Pasture/ Week	Percent of Feed Home Raised
ALL BOVINE	54.4	117.5	93.8	126.4	93.1
Beef Breeding	84.1	145.3	96.5	145.5	95.3
Calves	70.1	124.5	93.9	140.2	93.9
Yearlings	13.2	70.6	88.9	104.3	94.2
Feedlot Cattle	0.1	81.8	91.2	97.4	88.5
Miscellaneous	15.9	137.0	98.6	0.0[a]	0.0[a]
ALL PORCINE	0.1	18.4	78.6	67.1	63.8
Breeding Pigs	1.6	62.8	59.7	93.3	64.5
Baby Pigs	0.0	34.5	73.6	33.2	89.2
Fattening Pigs	0.0	11.5	81.1	74.9	53.7
ALL OVINE	46.0	147.4	90.9	122.0	89.4
Breeding Sheep	47.9	147.0	86.5	148.6	98.0
Fattening Sheep	43.9	147.7	95.8	44.3	64.3
ALL EQUINE	0.0	124.5	75.1	145.9	71.8
Breeding Horses	0.0	84.0	48.0	0.0[a]	0.0[a]
Foals	0.0	107.0	63.4	139.0	53.1
Yearlings	0.0	122.5	39.3	168.0	82.2
Pleasure Horses	0.0	126.3	77.9	145.7	72.5
ALL AVIAN	2.0	23.4	57.9	11.7	7.9
Chickens, layers	2.3	25.6	65.7	8.6	7.7
Chickens, broiler	0.0	0.0	16.8	8.4	0.0
Miscellaneous	2.5	50.4	37.6	33.5	23.3
Dogs (Canine)	4.1	78.7	20.0	121.3	8.5
Cats (Feline)	18.2	139.8	34.8	158.3	32.0

[a] There were no animals in these categories

TABLE 11-55. COMPARISON OF DURATION OF TIME SPENT ON FIELD, DURATION OF SLUDGE EXPOSURE, AND CONSUMPTION OF HOME GROWN FEED IN ANIMALS LIVING ON SLUDGE AND CONTROL FARMS, CLARK COUNTY

	Sludge			Control	
Species	No. of Hours of Exposure to Sludge Treated Fields /Week	No. of Hours Spent on Fields and Pasture/ Week	Percent of Feed Home Raised	No. of Hours Spent on Fields and Pasture/ Week	Percent of Feed Home Raised
ALL BOVINE	4.9	73.0	87.8	70.4	84.0
Beef Breeding	9.7	147.0	91.6	99.1	93.7
Calves	13.7	144.0	92.7	74.0	85.9
Yearlings	0.5	94.9	82.0	86.2	69.0
Feedlot Cattle	0.0	1.3	85.6	3.6	72.4
Miscellaneous	0.0[a]	0.0[a]	0.0[a]	0.0[a]	0.0[a]
ALL PORCINE	0.5	1.9	30.9	5.4	33.6
Breeding Pigs	3.1	10.8	42.1	31.9	70.9
Baby Pigs	0.0	0.0	41.7	0.0	42.8
Fattening Pigs	0.0	00.0	17.1	0.9	15.9
ALL OVINE	0.0	73.3	85.9	128.2	92.5
Breeding Sheep	0.0	110.3	97.7	137.3	97.0
Fattening Sheep	0.0	24.9	70.4	114.4	85.8
ALL EQUINE	0.0	104.1	72.3	0.0[a]	0.0[a]
Breeding Horses	0.0	76.3	93.2	0.0	0.0
Foals	0.0	118.6	98.5	0.0	0.0
Yearlings	0.0	111.2	93.4	0.0	0.0
Pleasure Horses	0.0	122.2	44.2	0.0	0.0
ALL AVIAN	0.3	80.9	44.6	76.0	47.8
Chickens, layers	0.3	56.7	39.4	56.5	49.6
Chickens, broiler	0.2	84.0	30.7	20.2	7.5
Miscellaneous	0.5	162.2	73.1	159.4	59.4
Dogs (Canine)	0.8	136.1	9.9	110.1	4.2
Cats (Feline)	0.6	155.2	27.4	156.2	27.7

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[a] There were no animals in these categories.

TABLE 11-56. COMPARISON OF ILLNESS RATES FOR SELECTED SIGNS OF ILLNESS IN SLUDGE AND CONTROL GROUPS IN ALL COUNTIES FOR ALL BOVINE

	Sludge					Control				
Signs	No. of Units Affected	No. of Episodes Reported	Rate per 100 Unit -Years at Risk	No. of Animals Affected	Rate Per 100 Animal-Years at Risk	No. of Units Affected	No. of Episodes Reported	Rate per 100 Unit -Years at Risk	No. of Animals Affected	Rate Per 100 Animal-Years at Risk
ALL REPORTED ILLNESSES[a]	30	62	69.0	207	13.9	35	82	75.4	360	20.0
Off Feed	15	16	17.8	88	5.9	12	21	19.3	141	7.8
Fever	7	7	7.8	59	4.0	7	11	10.1	103	5.7
Cough	4	4	4.5	51	3.4	6	7	6.4	94	5.2
Nasal Discharge	7	8	8.9	81	5.4	11	13	12.0	149	8.3
Difficult Breathing	4	5	5.6	46	3.1	15	18	16.5	151	8.4
Weakness	12	13	14.5	53	3.6	16	21	19.3	131	7.3
Abnormal Behavior	2	3	3.3	4	0.3	2	3	2.8	4	0.2
Constipation	1	1	1.1	1	0.1	0	0	0.0	0	0.0
Diarrhea	9	10	11.1	20	4.7	6	6	5.5	44	2.4
Blood in Feces	1	1	1.1	1	0.1	0	0	0.0	0	0.0
Sudden Death	3	3	3.3	3	0.2	3	3	2.8	6	0.3
Abortion	2	3	3.3	3	0.2	1	1	0.9	1	0.1

[a] Excludes 10 illness reports that were not related to sludge (due to accidents and chronic illnesses).

TABLE 11-57. COMPARISON OF ANIMAL ILLNESS RATES AND INCIDENCE RATES FOR SELECTED SIGNS OF ILLNESS IN SLUDGE AND CONTROL GROUPS IN MEDINA COUNTY FOR ALL BOVINE

	Sludge					Control				
Signs	No. of Units Affected	No. of Episodes Reported	Rate per 100 Unit -Years at Risk	No. of Animal-Episodes	Rate Per 100 Animal-Years at Risk	No. of Units Affected	No. of Episodes Reported	Rate per 100 Unit -Years at Risk	No. of Animal-Episodes	Rate Per 100 Animal-Years at Risk
ALL REPORTED ILLNESSES	6	7	50.3	8	7.6	4	4	19.7	4	3.1
Off Feed	2	2	14.4	3	2.8	3	3	14.8	3	2.3
Fever	0	0	0.0	0	0.0	1	1	4.9	1	0.8
Cough	0	0	0.0	0	0.0	1	1	4.9	1	0.8
Nasal Discharge	1	1	7.2	2	1.9	2	2	9.8	2	1.6
Difficult Breathing	0	0	0.0	0	0.0	2	2	9.8	2	1.6
Weakness	1	1	7.2	1	0.9	4	4	19.7	4	3.1
Abnormal Behavior	1	1	7.2	2	1.9	0	0	0.0	0	0.0
Constipation	1	1	7.2	1	0.9	0	0	0.0	0	0.0
Diarrhea	2	2	14.4	2	1.9	1	1	4.9	1	0.8
Blood in Feces	0	0	0.0	0	0.0	0	0	0.0	0	0.0
Sudden Death	0	0	0.0	0	0.0	0	0	0.0	0	0.0
Abortion	0	0	0.0	0	0.0	0	0	0.0	0	0.0

TABLE 11-58. COMPARISON OF ANIMAL ILLNESS RATES AND INCIDENCE RATES FOR SELECTED SIGNS OF ILLNESS IN SLUDGE AND CONTROL GROUPS IN FRANKLIN-PICKAWAY COUNTIES FOR ALL BOVINE

	Sludge					Control				
Signs	No. of Units Affected	No. of Episodes Reported	Rate per 100 Unit -Years at Risk	No. of Animal-Episodes	Rate Per 100 Animal-Years at Risk	No. of Units Affected	No. of Episodes Reported	Rate per 100 Unit -Years at Risk	No. of Animal-Episodes	Rate Per 100 Animal-Years at Risk
ALL REPORTED ILLNESSES[a]	17	39	62.7	114	10.6	20	54	83.9	127	10.1
Off Feed	8	9	14.5	47	4.4	5	11	17.1	20	1.6
Fever	4	4	6.4	18	1.7	3	6	9.3	66	5.2
Cough	2	2	3.2	33	3.1	1	1	1.6	3	0.2
Nasal Discharge	5	5	8.0	43	4.0	5	5	7.8	7	0.6
Difficult Breathing	2	2	3.2	9	0.8	6	9	14.0	62	4.9
Weakness	7	8	12.9	15	1.4	7	12	18.7	65	5.2
Abnormal Behavior	1	2	3.2	2	0.2	1	2	3.1	2	0.2
Constipation	0	0	0.0	0	0.0	0	0	0.0	0	0.0
Diarrhea	5	5	8.0	32	3.0	3	3	4.7	5	0.4
Blood in Feces	1	1	1.6	1	0.1	0	0	0.0	0	0.0
Sudden Death	2	2	3.2	2	0.2	3	3	4.7	6	0.5
Abortion	2	3	4.8	3	0.3	0	0	0.0	0	0.0

[a] Excludes 9 illnesses on report that were not related to sludge (due to accident and chronic illnesses).

TABLE 11-59. COMPARISON OF ANIMAL ILLNESS RATES AND INCIDENCE RATES FOR SELECTED SIGNS OF ILLNESS IN SLUDGE AND CONTROL GROUPS IN CLARK COUNTY FOR ALL BOVINE

	Sludge					Control				
Signs	No. of Units Affected	No. of Episodes Reported	Rate per 100 Unit -Years at Risk	No. of Animal-Episodes	Rate Per 100 Animal-Years at Risk	No. of Units Affected	No. of Episodes Reported	Rate per 100 Unit -Years at Risk	No. of Animal-Episodes	Rate Per 100 Animal-Years at Risk
ALL REPORTED ILLNESSES[a]	7	16	116.2	85	26.9	11	24	99.4	229	56.1
Off Feed	5	5	36.3	38	12.0	4	7	29.0	118	28.9
Fever	3	3	21.8	41	13.0	3	4	16.6	36	8.8
Cough	2	2	14.5	18	5.7	4	5	20.7	90	22.1
Nasal Discharge	1	2	14.5	36	11.4	4	6	24.9	140	34.3
Difficult Breathing	2	3	21.8	37	11.7	7	7	29.0	87	21.3
Weakness	4	4	29.1	37	11.7	5	5	20.7	62	15.2
Abnormal Behavior	0	0	0.0	0	0.0	1	1	4.1	2	0.5
Constipation	0	0	0.0	0	0.0	0	0	0.0	0	0.0
Diarrhea	2	3	21.8	36	11.4	2	2	8.3	38	9.3
Blood in Feces	0	0	0.0	0	0.0	0	0	0.0	0	0.0
Sudden Death	1	1	7.3	1	0.3	0	0	0.0	0	0.0
Abortion	0	0	0.0	0	0.0	1	1	4.1	1	0.2

[a] Excludes 1 illness on report that was not related to sludge (due to accident and chronic illness).

TABLE 11-60. COMPARISON OF ILLNESS RATES FOR SELECTED SIGNS OF ILLNESS IN SLUDGE AND CONTROL GROUPS IN ALL COUNTIES FOR ALL PORCINE

	Sludge					Control				
Signs	No. of Units Affected	No. of Episodes Reported	Rate per 100 Unit -Years at Risk	No. of Animals Affected	Rate Per 100 Animal-Years at Risk	No. of Units Affected	No. of Episodes Reported	Rate per 100 Unit -Years at Risk	No. of Animals Affected	Rate Per 100 Animal-Years at Risk
ALL REPORTED ILLNESSES[a]	21	73	168.7	967	28.5	33	138	230.4	1832	56.1
Off Feed	8	11	25.4	180	5.3	16	28	46.8	710	21.7
Fever	4	5	11.6	127	3.7	6	6	10.0	44	1.3
Cough	5	5	11.6	281	8.3	8	12	20.0	506	15.5
Nasal Discharge	2	2	4.6	2	0.1	4	4	6.7	418	12.8
Difficult Breathing	5	6	13.9	117	3.4	8	14	23.4	496	15.2
Weakness	14	23	53.2	359	10.6	18	35	58.4	431	13.2
Abnormal Behavior	2	2	4.6	2	0.1	1	1	1.7	3	.1
Constipation	2	2	4.6	20	0.6	1	1	1.7	1	0.0
Diarrhea	12	18	41.6	445	13.1	14	32	53.4	958	29.3
Blood in Feces	1	1	2.3	1	0.0	2	2	3.3	4	0.1
Sudden Death	7	10	23.1	14	0.4	7	10	16.7	28	0.9
Abortion	0	0	0.0	0	0.0	3	4	6.7	32	1.0

[a] Excludes 8 illness reports that were not related to sludge (due to accidents and chronic illnesses).

TABLE 11-61. COMPARISON OF ANIMAL ILLNESS RATES AND INCIDENCE RATES FOR SELECTED SIGNS OF ILLNESS IN SLUDGE AND CONTROL GROUPS IN MEDINA COUNTY FOR ALL PORCINE

	Sludge					Control				
Signs	No. of Units Affected	No. of Episodes Reported	Rate per 100 Unit -Years at Risk	No. of Animal-Episodes	Rate Per 100 Animal-Years at Risk	No. of Units Affected	No. of Episodes Reported	Rate per 100 Unit -Years at Risk	No. of Animal-Episodes	Rate Per 100 Animal-Years at Risk
ALL REPORTED ILLNESSES[a]	0	0	0.0	0	0.0	7	36	213.0	798	182.2
Off Feed	0	0	0.0	0	0.0	5	14	82.8	208	47.5
Fever	0	0	0.0	0	0.0	1	1	5.9	1	0.2
Cough	0	0	0.0	0	0.0	2	2	11.8	2	0.5
Nasal Discharge	0	0	0.0	0	0.0	1	1	5.9	1	0.2
Difficult Breathing	0	0	0.0	0	0.0	2	3	17.7	3	0.7
Weakness	0	0	0.0	0	0.0	4	12	71.0	256	58.4
Abnormal Behavior	0	0	0.0	0	0.0	1	1	5.9	3	0.7
Constipation	0	0	0.0	0	0.0	1	1	5.9	1	0.2
Diarrhea	0	0	0.0	0	0.0	3	16	94.7	769	175.5
Blood in Feces	0	0	0.0	0	0.0	1	1	5.9	3	0.7
Sudden Death	0	0	0.0	0	0.0	0	0	0.0	0	0.0
Abortion	0	0	0.0	0	0.0	1	2	11.8	4	0.9

[a] Excludes 4 illnesses on report that were not related to sludge (due to accident and chronic illnesses).

TABLE 11-62. COMPARISON OF ANIMAL ILLNESS RATES AND INCIDENCE RATES FOR SELECTED SIGNS OF ILLNESS IN SLUDGE AND CONTROL GROUPS IN FRANKLIN-PICKAWAY COUNTIES FOR ALL PORCINE

	Sludge					Control				
Signs	No. of Units Affected	No. of Episodes Reported	Rate per 100 Unit -Years at Risk	No. of Animal-Episodes	Rate Per 100 Animal-Years at Risk	No. of Units Affected	No. of Episodes Reported	Rate per 100 Unit -Years at Risk	No. of Animal-Episodes	Rate Per 100 Animal-Years at Risk
ALL REPORTED ILLNESSES[a]	10	33	126.5	380	19.3	15	74	237.8	430	21.6
Off Feed	2	3	11.5	52	2.6	8	11	35.4	99	5.0
Fever	2	3	11.5	8	0.4	3	3	9.6	37	1.9
Cough	3	3	11.5	157	8.0	3	5	16.1	55	2.8
Nasal Discharge	0	0	0.0	0	0.0	1	1	3.2	16	0.8
Difficult Breathing	2	3	11.5	8	0.4	4	7	22.5	47	2.4
Weakness	5	10	38.3	79	4.0	9	17	54.6	128	6.4
Abnormal Behavior	0	0	0.0	0	0.0	0	0	0.0	0	0.0
Constipation	0	0	0.0	0	0.0	0	0	0.0	0	0.0
Diarrhea	6	8	30.7	164	8.3	5	8	25.7	67	3.4
Blood in Feces	0	0	0.0	0	0.0	0	0	0.0	0	0.0
Sudden Death	4	4	15.3	5	0.3	5	8	25.7	24	1.2
Abortion	0	0	0.0	0	0.0	2	2	6.4	28	1.4

[a] Excludes 4 illnesses on report that were not related to sludge (due to accident and chronic illnesses).

TABLE 11-63. COMPARISON OF ANIMAL ILLNESS RATES AND INCIDENCE RATES FOR SELECTED SIGNS OF ILLNESS IN SLUDGE AND CONTROL GROUPS IN CLARK COUNTY FOR ALL PORCINE

	Sludge					Control				
Signs	No. of Units Affected	No. of Episodes Reported	Rate per 100 Unit -Years at Risk	No. of Animal-Episodes	Rate Per 100 Animal-Years at Risk	No. of Units Affected	No. of Episodes Reported	Rate per 100 Unit -Years at Risk	No. of Animal-Episodes	Rate Per 100 Animal-Years at Risk
ALL REPORTED ILLNESSES	11	40	274.8	587	41.6	11	28	236.0	604	72.0
Off Feed	6	8	55.0	128	9.1	3	3	25.3	403	48.0
Fever	2	2	13.7	119	8.4	2	2	16.9	6	0.7
Cough	2	2	13.7	124	8.8	3	5	42.1	449	53.5
Nasal Discharge	2	2	13.7	2	0.1	2	2	16.9	401	47 8
Difficult Breathing	3	3	20.6	109	7.7	2	4	33.7	446	53.1
Weakness	9	13	89.3	280	19.8	5	6	50.6	47	5.6
Abnormal Behavior	2	2	13.7	2	0.1	0	0	0.0	0	0.0
Constipation	2	2	13.7	20	1.4	0	0	0.0	0	0.0
Diarrhea	6	10	68.7	281	19.9	6	8	67.4	122	14.5
Blood in Feces	1	1	6.9	1	0.1	1	1	8.4	1	0.1
Sudden Death	3	6	41.2	9	0.6	2	2	16.9	4	0.5
Abortion	0	0	0.0	0	0.0	0	0	0.0	0	0.0

TABLE 11-64. COMPARISON OF ILLNESS RATES FOR SELECTED SIGNS OF ILLNESS IN SLUDGE AND CONTROL GROUPS IN ALL COUNTIES FOR ALL OVINE

	Sludge					Control				
Signs	No. of Units Affected	No. of Episodes Reported	Rate per 100 Unit -Years at Risk	No. of Animals Affected	Rate Per 100 Animal-Years at Risk	No. of Units Affected	No. of Episodes Reported	Rate per 100 Unit -Years at Risk	No. of Animals Affected	Rate Per 100 Animal-Years at Risk
ALL REPORTED ILLNESSES[a]	12	32	164.1	53	12.9	9	53	287.1	93	14.8
Off Feed	3	6	30.8	6	1.5	4	8	43.3	9	1.4
Fever	3	3	15.4	3	0.7	4	4	21.7	5	0.8
Cough	0	0	0.0	0	0.0	0	0	0.0	0	0.0
Nasal Discharge	1	1	5.1	1	0.2	1	2	10.8	2	0.3
Difficult Breathing	2	2	10.3	2	0.5	3	6	32.5	20	3.2
Weakness	5	9	46.2	12	2.9	6	17	92.1	50	7.9
Abnormal Behavior	0	0	0.0	0	0.0	3	3	16.3	4	0.6
Constipation	0	0	0.0	0	0.0	0	0	0.0	0	0.0
Diarrhea	1	1	5.1	1	0.2	0	0	0.0	0	0.0
Blood in Feces	0	0	0.0	0	0.0	0	0	0.0	0	0.0
Sudden Death	1	1	5.1	2	0.5	3	8	43.3	9	1.4
Abortion	1	1	5.1	1	0.2	1	2	10.8	4	0.6

[a] Excludes 10 illness reports that were not related to sludge (due to accidents and chronic illnesses).

TABLE 11-65. COMPARISON OF ANIMAL ILLNESS RATES AND INCIDENCE RATES FOR SELECTED SIGNS OF ILLNESS IN SLUDGE AND CONTROL GROUPS IN MEDINA COUNTY FOR ALL OVINE

	Sludge					Control				
Signs	No. of Units Affected	No. of Episodes Reported	Rate per 100 Unit -Years at Risk	No. of Animal-Episodes	Rate Per 100 Animal-Years at Risk	No. of Units Affected	No. of Episodes Reported	Rate per 100 Unit -Years at Risk	No. of Animal-Episodes	Rate Per 100 Animal-Years at Risk
ALL REPORTED ILLNESSES[a]	2	3	51.1	3	6.8	2	37	818.7	70	18.1
Off Feed	0	0	0.0	0	0.0	2	6	132.8	6	1.6
Fever	0	0	0.0	0	0.0	2	2	44.3	2	0.5
Cough	0	0	0.0	0	0.0	0	0	0.0	0	0.0
Nasal Discharge	0	0	0.0	0	0.0	1	2	44.3	2	0.5
Difficult Breathing	0	0	0.0	0	0.0	2	5	110.6	19	4.9
Weakness	0	0	0.0	0	0.0	2	11	243.4	37	9.6
Abnormal Behavior	0	0	0.0	0	0.0	2	2	44.3	2	0.5
Constipation	0	0	0.0	0	0.0	0	0	0.0	0	0.0
Diarrhea	0	0	0.0	0	0.0	0	0	0.0	0	0.0
Blood in Feces	0	0	0.0	0	0.0	0	0	0.0	0	0.0
Sudden Death	0	0	0.0	0	0.0	2	7	154.9	8	2.1
Abortion	0	0	0.0	0	0.0	1	2	44.3	4	1.0

[a] Excludes 1 illness on report that was not related to sludge (due to accident and chronic illness).

TABLE 11-66. COMPARISON OF ANIMAL ILLNESS RATES AND INCIDENCE RATES FOR SELECTED SIGNS OF ILLNESS IN SLUDGE AND CONTROL GROUPS IN FRANKLIN-PICKAWAY COUNTIES FOR ALL OVINE

	Sludge					Control				
Signs	No. of Units Affected	No. of Episodes Reported	Rate per 100 Unit -Years at Risk	No. of Animal-Episodes	Rate Per 100 Animal-Years at Risk	No. of Units Affected	No. of Episodes Reported	Rate per 100 Unit -Years at Risk	No. of Animal-Episodes	Rate Per 100 Animal-Years at Risk
ALL REPORTED ILLNESSES[a]	8	23	193.3	42	14.6	4	11	134.3	12	11.6
Off Feed	2	3	25.2	3	1.0	2	2	24.4	3	2.9
Fever	2	2	16.8	2	0.7	2	2	24.4	3	2.9
Cough	0	0	0.0	0	0.0	0	0	0.0	0	0.0
Nasal Discharge	1	1	8.4	1	0.3	0	0	0.0	0	0.0
Difficult Breathing	1	1	8.4	1	0.3	1	1	12.2	1	1.0
Weakness	3	5	42.0	6	2.1	3	4	48.8	5	4.8
Abnormal Behavior	0	0	0.0	0	0.0	1	1	12.2	2	1.9
Constipation	0	0	0.0	0	0.0	0	0	0.0	0	0.0
Diarrhea	1	1	8.4	1	0.3	0	0	0.0	0	0.0
Blood in Feces	0	0	0.0	0	0.0	0	0	0.0	0	0.0
Sudden Death	1	1	8.4	2	0.7	1	1	12.2	1	1.0
Abortion	1	1	8.4	1	0.3	0	0	0.0	0	0.0

[a] Excludes 5 illnesses on report that were not related to sludge (due to accident and chronic illnesses).

TABLE 11-67. COMPARISON OF ANIMAL ILLNESS RATES AND INCIDENCE RATES FOR SELECTED SIGNS OF ILLNESS IN SLUDGE AND CONTROL GROUPS IN CLARK COUNTY FOR ALL OVINE

	Sludge					Control				
Signs	No. of Units Affected	No. of Episodes Reported	Rate per 100 Unit -Years at Risk	No. of Animal-Episodes	Rate Per 100 Animal-Years at Risk	No. of Units Affected	No. of Episodes Reported	Rate per 100 Unit -Years at Risk	No. of Animal-Episodes	Rate Per 100 Animal-Years at Risk
ALL REPORTED ILLNESSES[a]	2	6	346.7	8	10.0	3	5	87.0	11	7.8
Off Feed	1	3	173.3	3	3.7	0	0	0.0	0	0.0
Fever	1	1	57.8	1	1.2	0	0	0.0	0	0.0
Cough	0	0	0.0	0	0.0	0	0	0.0	0	0.0
Nasal Discharge	0	0	0.0	0	0.0	0	0	0.0	0	0.0
Difficult Breathing	1	1	57.8	1	1.2	0	0	0.0	0	0.0
Weakness	2	4	231.1	6	7.5	1	2	34.8	8	5.7
Abnormal Behavior	0	0	0.0	0	0.0	0	0	0.0	0	0.0
Constipation	0	0	0.0	0	0.0	0	0	0.0	0	0.0
Diarrhea	0	0	0.0	0	0.0	0	0	0.0	0	0.0
Blood in Feces	0	0	0.0	0	0.0	0	0	0.0	0	0.0
Sudden Death	0	0	0.0	0	0.0	0	0	0.0	0	0.0
Abortion	0	0	0.0	0	0.0	0	0	0.0	0	0.0

[a] Excludes 4 illnesses on report that were not related to sludge (due to accident and chronic illnesses).

TABLE 11-68. COMPARISON OF ILLNESS RATES FOR SELECTED SIGNS OF ILLNESS IN SLUDGE AND CONTROL GROUPS IN ALL COUNTIES FOR ALL EQUINE

Signs	Sludge					Control				
	No. of Units Affected	No. of Episodes Reported	Rate per 100 Unit -Years at Risk	No. of Animals Affected	Rate Per 100 Animal-Years at Risk	No. of Units Affected	No. of Episodes Reported	Rate per 100 Unit -Years at Risk	No. of Animals Affected	Rate Per 100 Animal-Years at Risk
ALL REPORTED ILLNESSES[a]	5	5	19.6	5	5.6	5	6	21.8	6	10.4
Off Feed	2	2	7.8	2	2.3	0	0	0.0	0	0.0
Fever	1	1	3.9	1	1.1	0	0	0.0	0	0.0
Cough	0	0	0.0	0	0.0	1	1	3.6	1	1.7
Nasal Discharge	0	0	0.0	0	0.0	1	1	3.6	1	1.7
Difficult Breathing	2	2	7.8	2	2.3	0	0	0.0	0	0.0
Weakness	2	2	7.8	2	2.3	0	0	0.0	0	0.0
Abnormal Behavior	0	0	0.0	0	0.0	0	0	0.0	0	0.0
Constipation	0	0	0.0	0	0.0	0	0	0.0	0	0.0
Diarrhea	0	0	0.0	0	0.0	0	0	0.0	0	0.0
Blood in Feces	0	0	0.0	0	0.0	0	0	0.0	0	0.0
Sudden Death	0	0	0.0	0	0.0	0	0	0.0	0	0.0
Abortion	0	0	0.0	0	0.0	0	0	0.0	0	0.0

[a] Excludes 1 illness report that was not related to sludge (due to accident and chronic illness).

TABLE 11-69. COMPARISON OF ANIMAL ILLNESS RATES AND INCIDENCE RATES FOR SELECTED SIGNS OF ILLNESS IN SLUDGE AND CONTROL GROUPS IN MEDINA COUNTY FOR ALL EQUINE

Signs	Sludge					Control				
	No. of Units Affected	No. of Episodes Reported	Rate per 100 Unit -Years at Risk	No. of Animal-Episodes	Rate Per 100 Animal-Years at Risk	No. of Units Affected	No. of Episodes Reported	Rate per 100 Unit -Years at Risk	No. of Animal-Episodes	Rate Per 100 Animal-Years at Risk
ALL REPORTED ILLNESSES	1	1	17.8	1	13.5	0	0	0.0	0	0.0
Off Feed	0	0	0.0	0	0.0	0	0	0.0	0	0.0
Fever	0	0	0.0	0	0.0	0	0	0.0	0	0.0
Cough	0	0	0.0	0	0.0	0	0	0.0	0	0.0
Nasal Discharge	0	0	0.0	0	0.0	0	0	0.0	0	0.0
Difficult Breathing	0	0	0.0	0	0.0	0	0	0.0	0	0.0
Weakness	0	0	0.0	0	0.0	0	0	0.0	0	0.0
Abnormal Behavior	0	0	0.0	0	0.0	0	0	0.0	0	0.0
Constipation	0	0	0.0	0	0.0	0	0	0.0	0	0.0
Diarrhea	0	0	0.0	0	0.0	0	0	0.0	0	0.0
Blood in Feces	0	0	0.0	0	0.0	0	0	0.0	0	0.0
Sudden Death	0	0	0.0	0	0.0	0	0	0.0	0	0.0
Abortion	0	0	0.0	0	0.0	0	0	0.0	0	0.0

TABLE 11-70. COMPARISON OF ANIMAL ILLNESS RATES AND INCIDENCE RATES FOR SELECTED SIGNS OF ILLNESS IN SLUDGE AND CONTROL GROUPS IN FRANKLIN-PICKAWAY COUNTIES FOR ALL EQUINE

	Sludge					Control				
Signs	No. of Units Affected	No. of Episodes Reported	Rate per 100 Unit -Years at Risk	No. of Animal-Episodes	Rate Per 100 Animal-Years at Risk	No. of Units Affected	No. of Episodes Reported	Rate per 100 Unit -Years at Risk	No. of Animal-Episodes	Rate Per 100 Animal-Years at Risk
ALL REPORTED ILLNESSES	3	3	22.7	3	5.2	5	6	24.0	6	11.2
Off Feed	1	1	7.6	1	1.7	0	0	0.0	0	0.0
Fever	1	1	7.6	1	1.7	0	0	0.0	0	0.0
Cough	0	0	0.0	0	0.0	1	1	4.0	1	1.9
Nasal Discharge	0	0	0.0	0	0.0	1	1	4.0	1	1.9
Difficult Breathing	1	1	7.6	1	1.7	0	0	0.0	0	0.0
Weakness	1	1	7.6	1	1.7	0	0	0.0	0	0.0
Abnormal Behavior	0	0	0.0	0	0.0	0	0	0.0	0	0.0
Constipation	0	0	0.0	0	0.0	0	0	0.0	0	0.0
Diarrhea	0	0	0.0	0	0.0	0	0	0.0	0	0.0
Blood in Feces	0	0	0.0	0	0.0	0	0	0.0	0	0.0
Sudden Death	0	0	0.0	0	0.0	0	0	0.0	0	0.0
Abortion	0	0	0.0	0	0.0	0	0	0.0	0	0.0

TABLE 11-71. COMPARISON OF ANIMAL ILLNESS RATES AND INCIDENCE RATES FOR SELECTED SIGNS OF ILLNESS IN SLUDGE AND CONTROL GROUPS IN CLARK COUNTY FOR ALL EQUINE

	Sludge					Control				
Signs	No. of Units Affected	No. of Episodes Reported	Rate per 100 Unit -Years at Risk	No. of Animal-Episodes	Rate Per 100 Animal-Years at Risk	No. of Units Affected	No. of Episodes Reported	Rate per 100 Unit -Years at Risk	No. of Animal-Episodes	Rate Per 100 Animal-Years at Risk
ALL REPORTED ILLNESSES[a]	1	1	15.0	1	4.3	0	0	0.0	0	0.0
Off Feed	1	1	15.0	1	4.3	0	0	0.0	0	0.0
Fever	0	0	0.0	0	0.0	0	0	0.0	0	0.0
Cough	0	0	0.0	0	0.0	0	0	0.0	0	0.0
Nasal Discharge	0	0	0.0	0	0.0	0	0	0.0	0	0.0
Difficult Breathing	1	1	15.0	1	4.3	0	0	0.0	0	0.0
Weakness	1	1	15.0	1	4.3	0	0	0.0	0	0.0
Abnormal Behavior	0	0	0.0	0	0.0	0	0	0.0	0	0.0
Constipation	0	0	0.0	0	0.0	0	0	0.0	0	0.0
Diarrhea	0	0	0.0	0	0.0	0	0	0.0	0	0.0
Blood in Feces	0	0	0.0	0	0.0	0	0	0.0	0	0.0
Sudden Death	0	0	0.0	0	0.0	0	0	0.0	0	0.0
Abortion	0	0	0.0	0	0.0	0	0	0.0	0	0.0

[a] Excludes 1 illness on report that was not related to sludge (due to accident and chronic illness).

TABLE 11-72. COMPARISON OF ILLNESS RATES FOR SELECTED SIGNS OF ILLNESS IN SLUDGE AND CONTROL GROUPS IN ALL COUNTIES FOR ALL AVIAN

	Sludge					Control				
Signs	No. of Units Affected	No. of Episodes Reported	Rate per 100 Unit -Years at Risk	No. of Animals Affected	Rate Per 100 Animal-Years at Risk	No. of Units Affected	No. of Episodes Reported	Rate per 100 Unit -Years at Risk	No. of Animals Affected	Rate Per 100 Animal-Years at Risk
ALL REPORTED ILLNESSES[a]	10	30	79.0	120	12.7	13	29	115.4	124	19.3
Off Feed	1	1	2.6	1	0.1	1	1	4.0	1	0.2
Fever	0	0	0.0	0	0.0	0	0	0.0	0	0.0
Cough	0	0	0.0	0	0.0	0	0	0.0	0	0.0
Nasal Discharge	0	0	0.0	0	0.0	0	0	0.0	0	0.0
Difficult Breathing	1	2	5.3	46	4.9	0	0	0.0	0	0.0
Weakness	3	4	10.5	48	5.1	4	4	15.9	5	0.8
Abnormal Behavior	1	2	5.3	46	4.9	0	0	0.0	0	0.0
Constipation	0	0	0.0	0	0.0	0	0	0.0	0	0.0
Diarrhea	2	2	5.3	2	0.2	0	0	0.0	0	0 0
Blood in Feces	0	0	0.0	0	0.0	1	2	8.0	50	7.8
Sudden Death	4	5	13.2	8	0.8	7	11	43.8	17	2.6
Abortion	0	0	0.0	0	0.0	0	0	0.0	0	0.0

[a] Excludes 12 illness reports that were not related to sludge (due to accidents and chronic illnesses).

TABLE 11-73. COMPARISON OF ANIMAL ILLNESS RATES AND INCIDENCE RATES FOR SELECTED SIGNS OF ILLNESS IN SLUDGE AND CONTROL GROUPS IN MEDINA COUNTY FOR ALL AVIAN

	Sludge					Control				
Signs	No. of Units Affected	No. of Episodes Reported	Rate per 100 Unit -Years at Risk	No. of Animal-Episodes	Rate Per 100 Animal-Years at Risk	No. of Units Affected	No. of Episodes Reported	Rate per 100 Unit -Years at Risk	No. of Animal-Episodes	Rate Per 100 Animal-Years at Risk
ALL REPORTED ILLNESSES[a]	2	2	18.6	2	7.1	4	5	34.1	5	17.2
Off Feed	1	1	9.3	1	3.5	1	1	6.8	1	3.4
Fever	0	0	0.0	0	0.0	0	0	0.0	0	0.0
Cough	1	1	9.3	1	3.5	0	0	0.0	0	0.0
Nasal Discharge	0	0	0.0	0	0.0	0	0	0.0	0	0.0
Difficult Breathing	1	1	9.3	1	3.5	0	0	0.0	0	0.0
Weakness	0	0	0.0	0	0.0	0	0	0.0	0	0.0
Abnormal Behavior	0	0	0.0	0	0.0	0	0	0.0	0	0.0
Constipation	0	0	0.0	0	0.0	0	0	0.0	0	0.0
Diarrhea	1	1	9.3	1	3.5	1	1	6.8	1	3.4
Blood in Feces	1	1	9.3	1	3.5	1	1	6.8	1	3.4
Sudden Death	0	0	0.0	0	0.0	1	1	6.8	1	3.4
Abortion	0	0	0.0	0	0.0	0	0	0.0	0	0.0

[a] Excludes 1 illness on report that was not related to sludge (due to accident and chronic illness).

TABLE 11-74. COMPARISON OF ANIMAL ILLNESS RATES AND INCIDENCE RATES FOR SELECTED SIGNS OF ILLNESS IN SLUDGE AND CONTROL GROUPS IN FRANKLIN-PICKAWAY COUNTIES FOR ALL AVIAN

	Sludge					Control				
Signs	No. of Units Affected	No. of Episodes Reported	Rate per 100 Unit -Years at Risk	No. of Animal-Episodes	Rate Per 100 Animal-Years at Risk	No. of Units Affected	No. of Episodes Reported	Rate per 100 Unit -Years at Risk	No. of Animal-Episodes	Rate Per 100 Animal-Years at Risk
ALL REPORTED ILLNESSES[a]	6	18	88.0	35	5.3	7	18	173.0	57	20.9
Off Feed	1	1	4.9	1	0.2	0	0	0.0	0	0.0
Fever	0	0	0.0	0	0.0	0	0	0.0	0	0.0
Cough	0	0	0.0	0	0.0	0	0	0.0	0	0.0
Nasal Discharge	0	0	0.0	0	0.0	0	0	0.0	0	0.0
Difficult Breathing	0	0	0.0	0	0.0	0	0	0.0	0	0.0
Weakness	2	2	9.8	2	0.3	3	3	28.8	4	1.5
Abnormal Behavior	0	0	0.0	0	0.0	0	0	0.0	0	0.0
Constipation	0	0	0.0	0	0.0	0	0	0.0	0	0.0
Diarrhea	1	1	4.9	1	0.2	0	0	0.0	0	0.0
Blood in Feces	0	0	0.0	0	0.0	0	0	0.0	0	0.0
Sudden Death	3	4	19.6	5	0.8	4	7	67.3	13	4.8
Abortion	0	0	0.0	0	0.0	0	0	0.0	0	0.0

[a] Excludes 6 illnesses on report that were not related to sludge (due to accident and chronic illnesses).

TABLE 11-75. COMPARISON OF ANIMAL ILLNESS RATES AND INCIDENCE RATES FOR SELECTED SIGNS OF ILLNESS IN SLUDGE AND CONTROL GROUPS IN CLARK COUNTY FOR ALL AVIAN

	Sludge					Control				
Signs	No. of Units Affected	No. of Episodes Reported	Rate per 100 Unit -Years at Risk	No. of Animal-Episodes	Rate Per 100 Animal-Years at Risk	No. of Units Affected	No. of Episodes Reported	Rate per 100 Unit -Years at Risk	No. of Animal-Episodes	Rate Per 100 Animal-Years at Risk
ALL REPORTED ILLNESSES[a]	3	8	89.7	13	17.2	2	4	52.4	5	5.4
Off Feed	0	0	0.0	0	0.0	0	0	0.0	0	0.0
Fever	0	0	0.0	0	0.0	0	0	0.0	0	0.0
Cough	0	0	0.0	0	0.0	0	0	0.0	0	0.0
Nasal Discharge	0	0	0.0	0	0.0	0	0	0.0	0	0.0
Difficult Breathing	0	0	0.0	0	0.0	0	0	0.0	0	0.0
Weakness	0	0	0.0	0	0.0	0	0	0.0	0	0.0
Abnormal Behavior	0	0	0.0	0	0.0	0	0	0.0	0	0.0
Constipation	0	0	0.0	0	0.0	0	0	0.0	0	0.0
Diarrhea	0	0	0.0	0	0.0	0	0	0.0	0	0.0
Blood in Feces	0	0	0.0	0	0.0	0	0	0.0	0	0.0
Sudden Death	1	1	11.2	3	4.0	2	3	39.3	3	3.2
Abortion	0	0	0.0	0	0.0	0	0	0.0	0	0.0

[a] Excludes 3 illnesses on report that were not related to sludge (due to accident and chronic illnesses).

TABLE 11-76. COMPARISON OF ILLNESS RATES FOR SELECTED SIGNS OF ILLNESS IN SLUDGE AND CONTROL GROUPS IN ALL COUNTIES FOR DOGS

	Sludge					Control				
Signs	No. of Units Affected	No. of Episodes Reported	Rate per 100 Unit -Years at Risk	No. of Animals Affected	Rate Per 100 Animal-Years at Risk	No. of Units Affected	No. of Episodes Reported	Rate per 100 Unit -Years at Risk	No. of Animals Affected	Rate Per 100 Animal-Years at Risk
ALL REPORTED ILLNESSES[a]	11	14	17.9	17	10.3	21	30	44.5	32	25.2
Off Feed	2	2	2.6	2	1.2	8	9	13.4	9	7.1
Fever	0	0	0.0	0	0.0	5	5	7.4	5	3.9
Cough	2	2	2.6	2	1.2	1	1	1.5	1	0.8
Nasal Discharge	1	1	1.3	1	0.6	0	0	0.0	0	0.0
Difficult Breathing	1	1	1.3	1	0.6	3	3	4.5	3	2.4
Weakness	1	1	1.3	1	0.6	5	5	7.4	5	3.9
Abnormal Behavior	0	0	0.0	0	0.0	2	2	3.0	2	1.6
Constipation	1	1	1.3	1	0.6	0	0	0.0	0	0.0
Diarrhea	1	1	1.3	1	0.6	3	3	4.5	3	2.4
Blood in Feces	1	1	1.3	1	0.6	3	3	4.5	3	2.4
Sudden Death	1	1	1.3	1	0.6	1	1	1.5	1	0.8
Abortion	0	0	0.0	0	0.0	0	0	0.0	0	0.0

[a] Excludes 21 illness reports that were not related to sludge (due to accidents and chronic illnesses).

TABLE 11-77. COMPARISON OF ANIMAL ILLNESS RATES AND INCIDENCE RATES FOR SELECTED SIGNS OF ILLNESS IN SLUDGE AND CONTROL GROUPS IN MEDINA COUNTY FOR ALL DOGS

	Sludge					Control				
Signs	No. of Units Affected	No. of Episodes Reported	Rate per 100 Unit -Years at Risk	No. of Animal-Episodes	Rate Per 100 Animal-Years at Risk	No. of Units Affected	No. of Episodes Reported	Rate per 100 Unit -Years at Risk	No. of Animal-Episodes	Rate Per 100 Animal-Years at Risk
ALL REPORTED ILLNESSES[a]	1	4	46.6	72	33.2	4	7	98.6	62	22.3
Off Feed	0	0	0.0	0	0.0	1	1	14.1	1	0.4
Fever	0	0	0.0	0	0.0	0	0	0.0	0	0.0
Cough	0	0	0.0	0	0.0	0	0	0.0	0	0.0
Nasal Discharge	0	0	0.0	0	0.0	0	0	0.0	0	0.0
Difficult Breathing	1	2	23.3	46	21.2	0	0	0.0	0	0.0
Weakness	1	2	23.3	46	21.2	1	1	14.1	1	0.4
Abnormal Behavior	1	2	23.3	46	21.2	0	0	0.0	0	0.0
Constipation	0	0	0.0	0	0.0	0	0	0.0	0	0.0
Diarrhea	1	1	11.7	1	0.5	0	0	0.0	0	0.0
Blood in Feces	0	0	0.0	0	0.0	1	2	28.2	50	18.0
Sudden Death	0	0	0.0	0	0.0	1	1	14.1	1	0.4
Abortion	0	0	0.0	0	0.0	0	0	0.0	0	0.0

[a] Excludes 3 illnesses on report that were not related to sludge (due to accident and chronic illnesses).

TABLE 11-78. COMPARISON OF ANIMAL ILLNESS RATES AND INCIDENCE RATES FOR SELECTED SIGNS OF ILLNESS IN SLUDGE AND CONTROL GROUPS IN FRANKLIN-PICKAWAY COUNTIES FOR ALL DOGS

	Sludge					Control				
Signs	No. of Units Affected	No. of Episodes Reported	Rate per 100 Unit -Years at Risk	No. of Animal-Episodes	Rate Per 100 Animal-Years at Risk	No. of Units Affected	No. of Episodes Reported	Rate per 100 Unit -Years at Risk	No. of Animal-Episodes	Rate Per 100 Animal-Years at Risk
ALL REPORTED ILLNESSES[a]	9	12	23.8	15	14.2	12	20	47.1	22	27.8
Off Feed	1	1	2.0	1	0.9	6	7	16.5	7	8.8
Fever	0	0	0.0	0	0.0	4	4	9.4	4	5.0
Cough	1	1	2.0	1	0.9	1	1	2.4	1	1.3
Nasal Discharge	1	1	2.0	1	0.9	0	0	0.0	0	0.0
Difficult Breathing	0	0	0.0	0	0.0	2	2	4.7	2	2.5
Weakness	1	1	2.0	1	0.9	3	3	7.1	3	3.8
Abnormal Behavior	0	0	0.0	0	0.0	2	2	4.7	2	2.5
Constipation	1	1	2.0	1	0.9	0	0	0.0	0	0.0
Diarrhea	0	0	0.0	0	0.0	1	1	2.4	1	1.3
Blood in Feces	0	0	0.0	0	0.0	1	1	2.4	1	1.3
Sudden Death	1	1	2.0	1	0.9	0	0	0.0	0	0.0
Abortion	0	0	0.0	0	0.0	0	0	0.0	0	0.0

[a] Excludes 11 illnesses on report that were not related to sludge (due to accident and chronic illnesses).

TABLE 11-79. COMPARISON OF ANIMAL ILLNESS RATES AND INCIDENCE RATES FOR SELECTED SIGNS OF ILLNESS IN SLUDGE AND CONTROL GROUPS IN CLARK COUNTY FOR ALL DOGS

	Sludge					Control				
Signs	No. of Units Affected	No. of Episodes Reported	Rate per 100 Unit -Years at Risk	No. of Animal-Episodes	Rate Per 100 Animal-Years at Risk	No. of Units Affected	No. of Episodes Reported	Rate per 100 Unit -Years at Risk	No. of Animal-Episodes	Rate Per 100 Animal-Years at Risk
ALL REPORTED ILLNESSES[a]	0	0	0.0	0	0.0	5	5	49.0	5	26.8
Off Feed	0	0	0.0	0	0.0	1	1	9.8	1	5.4
Fever	0	0	0.0	0	0.0	1	1	9.8	1	5.4
Cough	0	0	0.0	0	0.0	0	0	0.0	0	0.0
Nasal Discharge	0	0	0.0	0	0.0	0	0	0.0	0	0.0
Difficult Breathing	0	0	0.0	0	0.0	1	1	9.8	1	5.4
Weakness	0	0	0.0	0	0.0	2	2	19.6	2	10.7
Abnormal Behavior	0	0	0.0	0	0.0	0	0	0.0	0	0.0
Constipation	0	0	0.0	0	0.0	0	0	0.0	0	0.0
Diarrhea	0	0	0.0	0	0.0	1	1	9.8	1	5.4
Blood in Feces	0	0	0.0	0	0.0	1	1	9.8	1	5.4
Sudden Death	0	0	0.0	0	0.0	0	0	0.0	0	0.0
Abortion	0	0	0.0	0	0.0	0	0	0.0	0	0.0

[a] Excludes 3 illnesses on report that were not related to sludge (due to accident and chronic illnesses).

TABLE 11-80. COMPARISON OF ILLNESS RATES FOR SELECTED SIGNS OF ILLNESS IN SLUDGE AND CONTROL GROUPS IN ALL COUNTIES FOR CATS

	Sludge					Control				
Signs	No. of Units Affected	No. of Episodes Reported	Rate per 100 Unit -Years at Risk	No. of Animals Affected	Rate Per 100 Animal-Years at Risk	No. of Units Affected	No. of Episodes Reported	Rate per 100 Unit -Years at Risk	No. of Animals Affected	Rate Per 100 Animal-Years at Risk
ALL REPORTED ILLNESSES[a]	13	24	50.2	76	31.9	22	43	82.3	68	25.1
Off Feed	3	3	6.3	4	1.7	7	8	15.3	12	4.4
Fever	0	0	0.0	0	0.0	1	1	1.9	2	0.7
Cough	4	5	10.5	21	8.8	3	3	5.7	4	1.5
Nasal Discharge	4	4	8.4	36	15.1	1	1	1.9	1	0.4
Difficult Breathing	4	5	10.5	38	15.9	3	4	7.7	7	2.6
Weakness	4	6	12.6	25	10.5	5	7	13.4	12	4.4
Abnormal Behavior	0	0	0.0	0	0.0	2	2	3.8	2	0.7
Constipation	0	0	0.0	0	0.0	0	0	0.0	0	0 0
Diarrhea	2	2	4.2	14	5.9	4	4	7.7	6	2.2
Blood in Feces	1	1	2.1	1	0.4	0	0	0.0	0	0.0
Sudden Death	1	1	2.1	5	2.1	8	9	17.2	11	4.1
Abortion	0	0	0.0	0	0.0	0	0	0.0	0	0.0

[a] Excludes 25 illness reports that were not related to sludge (due to accidents and chronic illnesses).

TABLE 11-81. COMPARISON OF ANIMAL ILLNESS RATES AND INCIDENCE RATES FOR SELECTED SIGNS OF ILLNESS IN SLUDGE AND CONTROL GROUPS IN MEDINA COUNTY FOR ALL CATS

	Sludge					Control				
Signs	No. of Units Affected	No. of Episodes Reported	Rate per 100 Unit -Years at Risk	No. of Animal-Episodes	Rate Per 100 Animal-Years at Risk	No. of Units Affected	No. of Episodes Reported	Rate per 100 Unit -Years at Risk	No. of Animal-Episodes	Rate Per 100 Animal-Years at Risk
ALL REPORTED ILLNESSES[a]	1	1	7.8	1	1.9	3	7	88.6	11	19.9
Off Feed	0	0	0.0	0	0.0	1	2	25.3	3	5.4
Fever	0	0	0.0	0	0.0	0	0	0.0	0	0.0
Cough	0	0	0.0	0	0.0	2	2	25.3	3	5.4
Nasal Discharge	0	0	0.0	0	0.0	1	1	12.7	1	1.8
Difficult Breathing	0	0	0.0	0	0.0	1	2	25.3	2	3.6
Weakness	0	0	0.0	0	0.0	1	3	38.0	4	7.2
Abnormal Behavior	0	0	0.0	0	0.0	1	1	12.7	1	1.8
Constipation	0	0	0.0	0	0.0	0	0	0.0	0	0.0
Diarrhea	0	0	0.0	0	0.0	1	1	12.7	1	1.8
Blood in Feces	0	0	0.0	0	0.0	0	0	0.0	0	0.0
Sudden Death	0	0	0.0	0	0.0	1	1	12.7	1	1.8
Abortion	0	0	0.0	0	0.0	0	0	0.0	0	0.0

[a] Excludes 1 illness on report that was not related to sludge (due to accident and chronic illness).

TABLE 11-82. COMPARISON OF ANIMAL ILLNESS RATES AND INCIDENCE RATES FOR SELECTED SIGNS OF ILLNESS IN SLUDGE AND CONTROL GROUPS IN FRANKLIN-PICKAWAY COUNTIES FOR ALL CATS

	Sludge					Control				
Signs	No. of Units Affected	No. of Episodes Reported	Rate per 100 Unit -Years at Risk	No. of Animal-Episodes	Rate Per 100 Animal-Years at Risk	No. of Units Affected	No. of Episodes Reported	Rate per 100 Unit -Years at Risk	No. of Animal-Episodes	Rate Per 100 Animal-Years at Risk
ALL REPORTED ILLNESSES[a]	10	19	67.5	43	32.3	11	21	69.6	30	23.5
Off Feed	3	3	10.7	4	3.0	3	3	9.9	5	3.9
Fever	0	0	0.0	0	0.0	0	0	0.0	0	0.0
Cough	3	4	14.2	17	12.8	0	0	0.0	0	0.0
Nasal Discharge	2	2	7.1	7	5.3	0	0	0.0	0	0.0
Difficult Breathing	2	3	10.7	9	6.8	0	0	0.0	0	0.0
Weakness	4	6	21.3	25	18.8	2	2	6.6	3	2.3
Abnormal Behavior	0	0	0.0	0	0.0	0	0	0.0	0	0.0
Constipation	0	0	0.0	0	0.0	0	0	0.0	0	0.0
Diarrhea	2	2	7.1	14	10.5	0	0	0.0	0	0.0
Blood in Feces	1	1	3.6	1	0.8	0	0	0.0	0	0.0
Sudden Death	1	1	3.6	5	3.8	5	5	16.6	6	4.7
Abortion	0	0	0.0	0	0.0	0	0	0.0	0	0.0

[a] Excludes 17 illnesses on report that were not related to sludge (due to accident and chronic illnesses).

TABLE 11-83. COMPARISON OF ANIMAL ILLNESS RATES AND INCIDENCE RATES FOR SELECTED SIGNS OF ILLNESS IN SLUDGE AND CONTROL GROUPS IN CLARK COUNTY FOR ALL CATS

	Sludge					Control				
Signs	No. of Units Affected	No. of Episodes Reported	Rate per 100 Unit -Years at Risk	No. of Animal-Episodes	Rate Per 100 Animal-Years at Risk	No. of Units Affected	No. of Episodes Reported	Rate per 100 Unit -Years at Risk	No. of Animal-Episodes	Rate Per 100 Animal-Years at Risk
ALL REPORTED ILLNESSES[a]	2	4	58.9	32	59.9	8	15	105.9	27	31.6
Off Feed	0	0	0.0	0	0.0	3	3	21.2	4	4.7
Fever	0	0	0.0	0	0.0	1	1	7.1	2	2.3
Cough	1	1	14.7	4	7.5	1	1	7.1	1	1.2
Nasal Discharge	2	2	29.5	29	54.3	0	0	0.0	0	0.0
Difficult Breathing	2	2	29.5	29	54.3	2	2	14.1	5	5.9
Weakness	0	0	0.0	0	0.0	2	2	14.1	5	5.9
Abnormal Behavior	0	0	0.0	0	0.0	1	1	7.1	1	1.2
Constipation	0	0	0.0	0	0.0	0	0	0.0	0	0.0
Diarrhea	0	0	0.0	0	0.0	3	3	21.2	5	5.9
Blood in Feces	0	0	0.0	0	0.0	0	0	0.0	0	0.0
Sudden Death	0	0	0.0	0	0.0	2	3	21.2	4	4.7
Abortion	0	0	0.0	0	0.0	0	0	0.0	0	0.0

[a] Excludes 7 illnesses on report that were not related to sludge (due to accident and chronic illnesses).

EPIDEMIOLOGY OF METAL RESIDUES AND INFECTIONS IN SLUDGE-EXPOSED LIVESTOCK

Chada S. Reddy, B.V.Sc., M.S., Ph.D.*
C. Richard Dorn, D.V.M., M.P.H.*
David N. Lamphere, D.V.M., M.S., Ph.D.*
Jean D. Powers, Ph.D.#
*Department of Veterinary Preventive Medicine
#Department of Veterinary Physiology and Pharmacology
The Ohio State University
Columbus, Ohio 43210

SUMMARY

Transmission of infectious agents and translocation of toxic heavy metals (cadmium, Cd; copper, Cu; lead, Pb; and zinc, Zn) from anaerobically digested sludge to farm animals grazing on sludge amended pastures and their tissues was studied. The rates of annual sludge application on the 8 study farms ranged from 2-10 dry metric tons per hectare and were determined to supply all the phosphorus requirements of the soil-plant system on these farms.

Annual sample collection and routine testing of livetock on these farms revealed no significant health risk associated with the possible presence of bacteria: Mycobacterium bovis, Salmonella spp., and common animal parasites including *Nematodirus spp.*, *Strongylus spp.*, *Strongyloides spp.*, *Trichuris spp.*, *Eimeria spp.*, *Ascaris spp.*, and *Ancylostomum spp.* in sludge. Annual tuberculin tests of calves that were slaughtered following 3 to 8 months of grazing on sludge applied pastures were negative.

Significantly higher fecal cadmium concentrations were detected in samples collected from cattle soon after being placed on sludge applied pastures as compared to pre-sludge values in the same animals. No increases were seen in samples from the control cattle or sludge exposed cattle late during the grazing period. Tissue analysis for toxic metals indicated significant Cd and Pb accumulations in kidneys of calves grazing sludge applied pastures compared to control calves. Muscle tissue showed no accumulation of any of the metals studied. Although older cows grazing sludge applied pastures had significantly higher blood Pb levels, no metal accumulation was seen in tissues. The results of this study showed no significant risk of animal infections associated with grazing of pastures amended with these low sludge application rates. Statistically significant accumulations of Cd and Pb in the kidney of calves grazing these pastures for

a relatively short period of time suggests that caution must be exercised to avoid prolonged exposures on pastures with high sludge application rates, especially for sludges with high concentrations of heavy metals.

INTRODUCTION

Land application of treated municipal sewage sludge is becoming increasingly popular as a method of sludge disposal because of both economic as well as resource reclamation considerations (Pahren, 1980; Young and Carlson, 1975). Hyde (1976) presented evidence for increased crop yield following moderate sludge applications. A Cooperative Extension Service-Ohio State University Bulletin (1982) contains data indicating the value of municipal sludges in replacing the commercial fertilizers to increase crop yields. Edds and Davidson (1981) showed that 19.8 tons/hectare of urban sludge would produce plant growth equivalent to that produced by recommended levels of commercial fertilizers. Sludges, however, contain microorganisms of communicable or zoonotic nature (e.g. bacteria, viruses, and parasites), toxic heavy metals and trace organics. If sludge application on farm land is not managed properly, these disease agents may affect animal and human health (Pahren *et al*., 1979).

A report by the WHO working group (WHO, 1981), following an extensive review and discussion of health risks associated with sludge-borne microbes, concluded that only two pathogens, the salmonellae and the eggs of *Taenia saginata*, need to be considered in a sludge application program on farm lands. Jelinek and Braude (1977) however, suggested that health risks from *Mycobacterium tuberculosis*, eggs of Ascarids and other parasites, and sludge-borne viruses are unknown and need to be evaluated. Sagik *et al*., (1980) reviewed data demonstrating the isolation of salmonellae from sludge as well as from animals fed sterile sludge spiked with known numbers of *Salmonella* organisms. Neither Sagik *et al*., (1980), nor Cliver (1980) were able to find reports demonstrating association between sludge-borne salmonellae and *Salmonella* infections in animals and/or man. Reilly *et al*., (1981), however, recently reported 26 incidents of human and animal salmonellosis in Scotland in which the infection originated from sewage and/or effluents either contaminating waterways or being applied on farmlands. In one of the three episodes associated with land application of sewage sludge, 200 people were affected after consuming raw milk from a dairy farm on which sludge was applied. During the same period, there were 5 known outbreaks of cysticercosis in Scotland attributed to the use of sludge on farmland (Macpherson *et al*., 1978; Reilly *et al*., 1981). Incidence of cysticercosis in cattle following sludge application on farmlands has also been reported from Australia (Rickard and Adolph, 1977) and the U.S. (Hammerberg, *et al*., 1978). It is likely that most or all of these episodes are a result of exposure to untreated sewage sludge.

Chaney (1980) pointed out that although sewage sludge has considerable quantities of trace elements, sludges containing industrial wastes with higher concentrations of toxic elements are of more concern. Consistent increases in soil concentrations of Zn, Cu and nickel (Ni) are known to occur as a result of land application of sludge (Page, 1974). Otte and LaConde (1977) observed increases in soil and plant concentrations of Cd, Zn, Cu and Ni in more than half of the 9 sites studied in the U.S., practicing land application of sludge. Phytotoxic concentrations of Zn in cheatgrass and dangerously high concentrations of Cd in wheat grain were observed at one site each. Hinesly et al., (1976) reported similar increases of Cd and Zn in soil and plant tissues but never in phytotoxic concentrations. Phosphorus levels in sludges appeared to be limiting the sludge loading rates and thus prevent excessive Cd and Zn accumulation. Although higher sludge (Cd) loading rates increased corn grain cadmium to levels higher than those reported as normal (Hinesly, et al., 1977), annual sludge application at rates just enough to provide supplemental nitrogen for corn resulted in Cd and Zn accumulations of no significant health risk. Furr et al., (1976a) concluded that Ni, Cd and mercury (Hg) are among the elements which significantly accumulated in certain garden crops grown on sludge amended soil. Hyde (1976) claimed good public acceptance of land application of sludge and no food chain hazards resulting from moderate (3-32 metric tons/ha) sludge applications, despite increased levels of Cd and Zn in soil and plant samples tested.

Although accumulation of Cd in animal muscle tissue is insignificant under conditions of normal soil Cd concentrations and plant uptake, significant elevation in kidney Cd of pheasants (Melsted et al., 1977), guinea pigs (Furr et al., 1976b), rats (Miller and Boswell, 1981) and swine (Hansen and Hinesly, 1979) fed grain or leaves from plants grown on soils amended with very high levels of sludge have been found. The possible Cd entry into poultry and egg products is suggested by the report of Leach et al. (1979). Significant accumulations of Cd and Zn in liver and kidney and Cu and lead (Pb) in the liver were reported in cows and calves that grazed on pastures irrigated with liquid sludge (Fitzgerald, 1980). The exposure in the case of calves was for less than a year (Fitzgerald, 1980) and for this reason, the magnitude of accumulation of toxic metals was lower compared to that of cows. Decker et al., (1980) noted poor performance, and iron accumulation in liver, spleen and intestines of steers grazing on pastures that were sprayed with high iron sludge. Long term feeding of sludge as a part of animal diets resulted in accumulation of Cd in liver and kidney of cattle and swine as well as suppression of growth and reproductive performance in swine (Edds, et al., 1980). Liver and kidney from these animals, when fed to mice as 5% of their diet, caused translocation of Cd and Pb into mouse tissues and also caused reproductive impairment (Edds et al., 1980). Boyer et al., (1981) also reported increases in Pb and Cd in kidney, and Pb, Cd and Cu in the liver of steers fed Denver sewage sludge at 12% level in their diet. Dorn (1979) reviewed the available evidence and pointed out the possible contribution of sewage sludge to already high daily human cadmium intake by way of its translocation via sludge-soil-plant-animal human chain and also a direct plant-human chain. Following a careful review of available literature, the Council for Agricultural Science and Technology (CAST, 1976) recognized Cd, Cu, Pb and Ni as the elements of major environmental concern. Robens (1980) added Zn and molybdenum (Mo) to this list.

No comprehensive study of animals on farms receiving relatively low level municipal sewage sludge application has been conducted. Therefore the research reported here was undertaken to study the potential animal and human health affects resulting from land application of treated sewage sludge applied at rates pre-determined to be beneficial to soil and agricultural production on Ohio farms. This report summarizes measurements of the numbers of selected fecal parasite ova and of the frequency of Salmonella isolations in feces of farm animals and pets on a sample of 8 farms. The study also evaluates the extent of exposure of cattle to Mycobacterium spp and the extent of heavy metal translocation from sludge into cattle tissues.

METHODS

Eight farms were selected based on the presence of cattle (a breeding herd of at least 10 cows) that must be pastured a substantial part of the year, suitability of the pasture for sludge application, farmer willingness to supply four calves each year for slaughter, and a willingness by the farmer to cooperate during testing and sampling of animal herds. There were 18 eligible cattle farms in Pickaway County and 3 eligible cattle farms in Franklin County. Therefore the 8 cattle farms selected comprised a 38 percent sample of the eligible farms.

Chronology of herd testing, sludge application and sampling. Four farms were recruited in the fall of 1978 (Fall farms) and four more in the spring of 1979 (Spring farms). Each farm was randomly assigned into either the sludge-receiving group or the control group. All cattle on the four sludge receiving farms were tested for tuberculosis (caudal fold test) within two weeks before sludge application. At this time, individual fecal and hair samples were collected from all cattle. Four calves (1-2 months of age) were identified by ear tags and 300 ml of blood was collected from the jugular vein into heparinized polyethylene bottles. These calves were allowed to graze for 3-8 months on sludge applied pasture before they were slaughtered. Composite fecal samples from all other food producing animals on the farm, and individual fecal samples of horses and pets were also collected for Salmonella isolation and parasitological examination. Up to five individual cattle fecal samples were composited for Salmonella isolation and parasitological examination. Individual cattle fecal samples were dried, percentage dry matter calculated, ground to a fine powder with a porcelain mortar and pestle and stored in acid-washed polyethylene bottles for heavy metal analysis. Hair samples were classified into 9 color coded color categories ranging from blonde to black, washed twice with a commercial detergent (Snoop, 1%, Nupro Co., Willoughby, OH), rinsed thoroughly in double distilled deionized water, air dried and stored in plastic bags (Whirlpack, Pioneer Container Corp., Cederburg, WI) until analyzed for heavy metals. Fecal samples from study calves were analyzed for Cd, Cu, Pb and Zn whereas those from other cattle were analyzed for Cd only.

Within 2 to 3 weeks following the initial herd testing, sludge was applied on the farms at rates of 2-10 dry m. tons/hectare (Cooperative Extension Service-The Ohio State University guide, 1979). Average concentrations of Cd, Cu, Pb and Zn in the sludge applied on these farms for the period 1978-1982 were 77.8, 733, 557 and 5223 mg/kg respectively. Following a 30 days witholding period, cattle were allowed to graze on the pastures for a period ranging from 5 to 8 months. Within two weeks from the beginning of grazing, fresh fecal samples (pasture pick ups) from the grazing cattle were collected directly from the pastures for Salmonella isolation, parasitologic examination and heavy metal analysis.

At the end of 3-8 month grazing period, four calves (identified at the initial testing) from each farm were tuberculin tested, using comparative cervical test, and then slaughtered. Feces, blood, hair, liver, kidney, muscle (triceps) and bone (12th rib) samples from these animals were subjected for heavy metal analysis. Fecal samples were examined for Salmonella and parasites. Gross examinaton of all carcasses for abnormalities was routinely conducted.

Similar herd testing and calf slaughter was conducted annually for two additional years. Control farms were also tested and sampled on an annual basis using the same procedures used on the sludge-receiving farms. The chronology of herd testing-sampling and calf slaughter are shown in Figure 12-1. As indicated in Figure 12-1, the number of sludge applications on these farms ranged from 2 to 6 and were dependent upon weather, soil type, and the accessibility of the fields to hauling equipment. In addition, a ban on sludge hauling was instituted in the summer of 1980 in Pickaway county by the local health department due to negative reaction from a number of individuals resulting from inadvertant hauling of untreated, odorous sewage sludge by a sludge hauler. Therefore, 3 of the 4 sludge group farms received no additional sludge after the summer of 1980 (Figure 12-1).

In addition, when older cows from the study farms destined for slaughter were available, samples were collected for Salmonella isolation, parasitology and heavy metal analysis. Ten cows from sludge farms and two cows from control farms were available for this testing.

Tuberculosis testing

Caudal fold test: All cattle participating in the project were identified by metal ear tags and tested according to USDA Uniform Methods and Rules for tuberculin testing USDA (1982). The test consisted of intradermal injection of 0.1 ml of tuberculin (Tuberculin Purified Protein Derivative [PPD] Bovis Intradermic, produced for Animal and Plant Health Inspection Service, United States Department of Agriculture) in the caudal fold at the base of the tail. Seventy two hours later, the site of injection was examined visually as well as by palpation for diffuse or circumscribed swelling. A diffuse reaction of increased skin thickness twice or more of that of normal skin or a circumscribed enlargement of 5 mm or more was considered to be a positive response.

Cervical test: All of the calves sampled and slaughtered from each of the 8 cattle study farms were tuberculin tested using human (Connaught Laboratories Ltd., Willowdale, Ontario, Canada), bovine (USDA) and avian (USDA) at three different sites on the neck. The U.S. Department of Agriculture (USDA) procedures for cervical testing were followed (USDA, 1973). Testing was performed over a 3 year period yielding a total of 40 tests on calves from sludge farms and 40 tests on calves from control farms. The cervical testing was initiated starting with the second group of calves; therefore, the first 8 calves from sludge farms and first 8 calves from control farms were tested by the caudal fold method instead of the cervical method. The standardized tuberculins (0.1 ml) were injected into separate 4 square inch clipped sites in the upper cervical area of cattle. The normal skin thickness was measured with calipers. At 72 hr following injection, the response was recorded as the increase (mm) in post-injection skin thickness as compared to pre-injection thickness. Increase in thickness was plotted on the USDA report form VS-Form 6-22D. According to the USDA procedures, increases of 2.75 to 4.74 mm and greater than 4.75 mm of skin thickness at the bovine site with no concurrent reaction at the avian site are required for 'suspect' and 'reactor' classifications, respectively.

Salmonella Isolation and Identification

Composite fecal samples from cattle and other production animal units, and individual samples from equines and pets were cultured for Salmonella. Isolation of salmonellae was attempted by lightly swabbing feces directly onto differential and selective media (MacConkey, XLD, Hektoen enteric, and bismuth sulfite agars). To enhance the chances of recovering salmonellae present in low numbers in comparison with coliforms and other enteric bacteria, 10 ml of selenite enrichment broth was inoculated with approximately 1 gram of feces and incubated for 1 to 2 days at 37°C. Then a loopful of selenite broth culture was streaked onto each of the media described above.

Plates were incubated for 18 to 24 hours at 37°C and then examined for the presence of suspicious Salmonella colonies. Suspect colonies were picked to inoculate triple sugar iron agar (TSI) slants and urea agar slants and incubated for 24 hours. Antiserum for Salmonella somatic O antigen groups A through I was used to perform a slide agglutination test on each isolate giving an alkaline slant and an acid butt on TSI, regardless of H_2S production, and a negative reaction on urea agar. Isolates showing agglutination were subjected to a battery of differential biochemical tests (API 20E strips, Analytab Products, Plainview, NY) to determine whether their characteristics were consistent with those recognized for members of the genus Salmonella. All positive isolates were confirmed and serotyped by the Ohio Department of Health Laboratory, Columbus, Ohio.

Parasitologic examination

Individual samples from horses, pets and calves identified for slaughter, and composite samples from the rest of the cattle herds and other production units were subjected to parasitological examination by the McMaster's technique (Georgi, 1974). The technique involves weighing 2 g of wet feces, suspending them in 28 ml of saturated sodium nitrate, taking up the suspension into a sieve pipette (Whitlock and Co., New South Wales, Australia), filling one of the three chambers of the McMaster's slide (0.3 ml/chamber) and examining under low power of a microscope. Quantitative evaluation of the ova of Nematodirus, Strongyle, Strongyloides, Trichuris and Eimeria spp were done on all individual cattle samples. In addition, the canine samples were specifically examined for ova of Ascaris sp. in swine, and Ancylostomum sp. (hookworm). All equine, pet animal and composite cattle fecal samples were evaluated by floatation technique (Georgi, 1974).

Post mortem examination for Taenia saginata cysticercosis, the intermediate stage of the human tapeworm, Taenia saginata was performed on each of the 48 calves from sludge farms and 48 calves from control farms that were slaughtered. In addition, 12 cull cows were similarily examined. This examination consisted of examination of the masseter, diaphram, triceps and cardiac muscles for presence of dead or live cysts.

Heavy metal analysis

Analyses were done on a double beam atomic absorption spectrophotometer (Model AA-775, Varian Techtron Pty. Ltd., Springvale, Australia) equipped with a deuterium lamp for simultaneous background correction for nonatomic absorption. Filter-purified water of 18 megaohm conductivity (Milli-Q Model MQ2, Millipore Corp., Bedford, MA) was used throughout the experiment for final glassware rinsing, sample processing and reagent preparation. Laboratory glassware was washed 3 times with a caustic laboratory detergent (Event, Economics Laboratory, Inc., St. Paul, MN) and rinsed with distilled water in a mechanical dishwasher. Washed glassware and new sample containers were rinsed 3 times in filter-purified water, soaked overnight in 10% reagent grade nitric acid and given 3 final rinses with filter-purified water before being oven dried (glassware) or air dried (plastic-ware).

Concentrated redistilled nitric acid (G. F. Smith Chemical Co., Columbus, OH) was added to duplicate samples in 30 ml Kjeldahl flasks. The samples were placed in aluminum heating blocks, located in a fume hood, and brought to a gentle boil (120-130°C). Varying amounts (0.5 to 2.0 ml depending on the type of tissue) of 30% hydrogen peroxide (H_2O_2, Mallinkrodt) were added to each sample, approximately 24 hr. before acid evaporation. Samples were cooled prior to H_2O_2 addition, to prevent sample spillage from potentially explosive evolution of O_2 from the acid-peroxide mixture. Heating for an additional 24 hr. was necessary for decolorization and complete sample digestion. The acid was then evaporated to near dryness at 180 to 200°C. The residue was dissolved in a constant volume of deionized water or 5% HNO_3 and the digested samples were stored in sealed 1 oz. polyethylene bottles (Nalge Co., Division of Sybron Corp., Rochester, NY) until analyzed.

Analytical results were calculated and recorded as μg/100 ml for blood and as μg/g either on a wet basis for liver, kidney cortex and muscle or on "as is" basis for hair, and dry weight basis for feces.

The method of standard additions was used to calibrate atomic absorption readings for each sample type. Duplicate spikes of 0%, 50%, 100% and 150% of the samples's expected content of each analyte element were prepared for a standard additions series. These samples were digested, diluted and analyzed along with the other samples of the same tissue.

Detection limits were defined as sample analyte element concentrations equal to twice the standard deviation for replicate absorbance readings of a low concentration sample. Standard deviations for detection limit determinations were calculated from series of 10 repeated absorbance readings of relatively low concentration samples taken against the samples' blanks.

Feces:

Duplicate 200 mg samples of dried cattle feces in 30 ml Kjeldahl flasks were added with 10 ml of concentrated HNO_3. After 24 hr of digestion, 0.5 ml of H_2O_2 was added to each sample and they are reheated for an additional 24 hr period. Acid was evaporated to near dryness and the residue dissolved in 25 ml of deionized water. Copper and Zn were analyzed using air-acetylene flame atomization (FA), and Cd and Pb were analyzed using carbon furnace (rod) atomization (CRA).

Liver and Kidney:

Duplicate 0.5 mg samples were digested in 10 ml of concentrated HNO_3 for 48 hr. At 24 hr, 2 ml and 0.5 ml of H_2O_2 were added to liver and kidney samples, respectively. After evaporation to near dryness, the samples were diluted to a final volume of 25 ml with deionized water.

Copper and Zn were analyzed using FA, and Cd and Pb were analyzed using CRA methods. An absorbance expansion factor (AEF) of 2 was used for copper in both tissues and Pb in kidney. Cadmium in kidney required a 50% dilution (with 5% HNO_3) and an absorbance attenuation of 0.333.

Muscle:

Duplicate 1.5 g samples of triceps were digested in 15 ml of conc. HNO_3 for 96 hr. At 72 hr, 1 ml of H_2O_2 was added. Final dilution volume was 10 ml with deionized water.

Copper and Zn were analyzed using FA, and Cd and Pb were analyzed using CRA. An AEF of 2 was used for copper. Lead levels were below detection limit in all samples.

Bone:

Approximately 1 g duplicate samples of the 12th rib were digested in 10 ml of concentrated HNO_3, as described for muscle. Analysis of Zn was performed using FA, and Cd, Cu and Pb was done using 50% dilution (with 3% HNO_3) of the digestate and CRA.

Hair:

Duplicate 0.5 g samples were digested in 15 ml of concentrated HNO_3 for 72 hr. At 48 hr, 2 ml of H_2O_2 was added. The samples were taken to a final volume of 10 ml with 5% HNO_3.

Copper and Zn were analyzed using FA, and Cd and Pb were analyzed using CRA. An AEF of 2 for Cu, and absorbance attenuation of 0.5 for Cd were used. Final dilution of the digestate with 5% HNO_3 was necessary for repeatability in Pb analysis.

Blood:

Duplicate 5 ml samples of blood were each added to 10 ml of concentrated HNO_3 and left at room temperature, overnight, in a fume hood. They were then gradually heated in the aluminum blocks, cooling the flasks when excessive foaming occurred. When there was no further foaming, samples received an additional 5 ml of concentrated HNO_3 and were allowed to digest for 24 hr. After cooling to room temperature, 2 ml of peroxide was added to each sample and heating continued for 24 hr. The samples were evaporated to approximately 1 ml, and adjusted to a final volume of 10 ml.

Copper and Zn were analyzed using FA, and Cd and Pb were analyzed using CRA. An AEF of 2 was used for Pb analysis. Most samples had Cd levels below detection limit.

Data Analysis

The period of observation and testing before first sludge application is referred to as pre-sludge period. The period following the first sludge application ending with the last day of data collection is referred to as the post-sludge period. The frequency distributions of the observed concentrations of each element in each type of sample were plotted and then compared with similar plots after log transformation of the data. The log transformed data usually more closely approximated the normal distribution than the non-transformed data and was therefore used in the analysis of variance procedures. Heavy metal concentrations were compared between pre-sludge and post-sludge fecal, hair and blood samples within the two treatment group (sludge receiving farms and control farms) and between treatment groups within each period (pre- and post-sludge). Post-sludge period on control farms represents grazing period comparable to that on the matched sludge-applied farms. The Statistical Analysis System (SAS) general linear models procedure was executed through The Ohio State University - Instructional and Research Computer Center. The mean square for farms nested within treatment was used as an error term to evaluate the treatment effects, and mean square for farms' interaction with period (pre- or post-sludge) nested within treatment was used as an error term to evaluate the period effect.

Comparisons of heavy metal concentrations in other tissues between the treatment groups was also done by analysis of variance using the mean square for farms nested within treatment as an error term. For a heavy metal value that was below the detection limit for that element, a value of half the detection limit was used in statistical analysis.

RESULTS

Number of cattle contributing each type of sample and testing are indicated in the discussion of each sample type. Breeds represented by these cattle were: Hereford, Charolais, Simmental, Angus, Holstein and Santa Gertrudis.

Tuberculosis testing

Caudal fold test: A total of 452 sludge exposed animals and 241 control animals tested for tuberculosis one or more times. No positive reactions were noted in either group. The numbers of animals tested and the numbers of tests performed are summarized in Table 12-1.

Comparative cervical test: None of the reactions at the human PPD injection site was greater than 2.75 mm. Three sludge exposed animals and one control animal showed greater than 2.75 mm at the avian tuberculin injection site. None of these reactions, however, were greater than 4.75 mm (data not shown). None of the 40 calves in the sludge group demonstrated a reaction greater than 2 mm to the bovine PPD (Table 12-2). One animal in the control group with a 3 mm response to bovine PPD also had a 4 mm response to avian PPD.

Salmonella isolation

A total of 146 fecal samples from sludge exposed animals and 138 samples from control animals were screened for the presence of *Salmonella* organisms. The animal species present on the farms and the number of samples screened representing each species are listed in Table 12-3. A total of two samples from animal populations on sludge-receiving farms and 4 from control farms, were found to be positive (Table 12-4). All isolates from control farms were *S. montevideo.* These isolations involved two cattle, one horse and one swine. One of the two isolates from the sludge exposed cattle was *S. montevideo* and the second isolate was the serotype *S. muenchen.* *S. muenchen* was also isolated from a child living on the farm one year prior to *S. muenchen* isolation from cattle. Weekly analysis of samples of sludge that was applied on these farms revealed *S. St. Paul* in a sample collected 4 months prior to the isolation of *S. muenchen* from cattle. All other sludge samples, representing the weeks of sludge application on all experimental farms, were negative for salmonellae.

Parasitologic observations

A comparison of the number of cattle fecal samples processed in each of the study groups and the mean counts and ranges of parasite ova representing both pre-sludge and post-sludge periods, are presented in Table 12-5. Descriptive evaluation of the data in Table 12-5 revealed no apparent pattern in post-sludge increases in the load of fecal parasite ova in cattle on sludge receiving farms compared to samples from animals on control farms during a comparable period. The increases in the numbers of strongyle ova during the 2-3 years post-sludge period and strongyloides ova during the 1-2 years post-sludge period, were not associated with corresponding increases in samples from control animals. Only one of four sludge samples examined for parasites in our laboratory contained small numbers of ova of *Ascaris, Ancylostomum* and *Eimeria* species (data not shown). Examination of weekly sludge samples by the Department of Medical Microbiology yielded negative results (Chapter 16). None of the 96 calves or 12 culled cows from either sludge applied or control farms exhibited lesions indicative of cysticercosis at the time of slaughter.

Translocation of heavy metals from sludge to cattle tissues

A summary of the detection limits for various heavy metals in various sample matrices is presented in table 12-6.

Feces. A comparison of the fecal heavy metal concentrations from calves

(Table 12-7) indicates a significant decrease Zn concentrations in post-sludge fecal samples from control calves compared to pre-sludge samples from the same animals. Cadmium, Cu and Pb concentrations also showed a similar tendency. No differences were noted in sludge-exposed calves. However, there was a tendency for higher fecal Cd concentrations following sludge exposure.

No apparent differences were noted between the calves representing the two study groups before grazing (pre-sludge period) for all elements. Following grazing, calves and cows on sludge applied pastures tended to have higher fecal Cd, Cu, Pb and Zn concentrations compared to calves and cows on control pastures (Tables 12-7, 12-8), but these differences were not statistically significant.

Fifty eight cows from sludge-receiving farms and 35 cows from control farms remained on these farms for the entire project period. Chronological analysis of fecal cadmium concentrations indicated a trend similar to that seen in calves (Table 12-9). A tendency for a consistent rise in fecal Cd concentrations was seen in sludge-exposed cows each year following sludge application whereas the levels tended to drop in control cow samples with each successive year (Table 12-9).

Since the annual fecal sample collection during herd testing, as well as the fecal sampling at slaughter was preceded by withdrawal of the animals from pastures for varying lengths of time, it was decided to look at the heavy metal concentrations in feces collected while the animals were grazing the experimental pastures (pasture pick-up samples). This phase of the project was limited to the sampling following first sludge application. Cattle on one of the 4 sludge-receiving farms were away from the sludge applied pasture at the time of sampling. For this reason only the pasture pick-up samples from 3 remaining farms and their matching control farms were used in this comparison. These results were compared with those of fecal samples obtained during the initial herd testing, to evaluate the effect of sludge.

The results presented in Table 12-10 indicate that within the sludge exposed cattle, fecal Cd concentrations following grazing on sludge applied pastures were significantly higher compared to those in feces before sludge application ($P < 0.01$). In cattle grazing on control pastures, however, a significant decrease in Cd concentration was observed (Table 12-10) in samples collected in the post-sludge period compared to those collected during the pre-sludge period. Although fecal cadmium concentrations in cattle grazing sludge applied pastures were 2.5 times higher than those in feces from control cows, the differences were not statistically significant at the 5% level. Copper concentrations in pasture pick up samples from sludge-exposed cattle also tended to be higher than the concentrations in samples from control cattle.

Liver. The mean liver concentration (wet weight) of Cd in calves grazing sludge applied pasture were almost twice as high as those of calves from control pastures (Table 12-11). Copper, on the other hand, was twice as high in the control calf livers as was found in calves grazing sludge applied pastures. These differences, however, were not statistically significant. No apparent differences were found, in the Pb and Zn levels, between the 2 groups of calves.

Cd levels in livers from culled cows on sludge and control farms were 2 and 4 times as high as those in calves, respectively. No significant differences were noted between the sludge and control group cows for any of the heavy metals concentrations in liver (Table 12-12). Liver copper levels, again, were twice as high in control animals as were found in cows exposed to sludge.

Kidneys. Significantly higher concentrations of Cd and Pb were seen in kidney cortex from sludge exposed calves compared to those in controls. No apparent differences were found either in Cu and Zn levels in calf kidneys or any of the elements in the kidneys of culled cows, between the two groups (Tables 12-13, 12-14).

Muscle (Triceps). Lead concentrations in all samples were below detection limit (0.032 μg/g). None of the other elements, (Cd, Zn, Cu), showed any significant accumulations in this tissue of either the calves or the culled cows (Tables 12-15, 12-16).

Bone. Although there was a tendency for higher bone Pb concentrations in sludge-exposed calves (Table 12-17) and a tendency for higher bone Cu concentrations in control culled cows (Table 12-18) compared to those in control calves and sludge-exposed cows, respectively, no significant differences were noted in concentrations of any elements in bone of either calves or cows between the two groups.

Hair. Pre-sludge hair samples from calves on sludge and control farms contained similar concentrations of all 4 elements. Among post-sludge samples, a tendency for higher levels of Zn and lower levels of Cu in the hair of sludge-exposed calves compared to those of control calves, was found (Table 12-19). Within each study group, the post-sludge concentrations of Cd and Cu were lower ($P < 0.05$) compared to the pre-sludge samples. Zinc concentration significantly ($P < 0.05$) increased following grazing on sludge applied pastures as compared to control pastures. No significant differences were found in the heavy metal concentrations of post-sludge hair samples from older cows (Table 12-20). Analysis of heavy metal concentrations by differences in the hair color failed to indicate any consistent trend.

Blood. Most Cd concentrations were below the detection limit of 0.022 μg/100 ml for these samples, no differences were noted in blood Cd levels of calves either between treatments within each period (pre- and post-sludge) or between periods within each study group (Tables 12-21, 12-22). In cows, blood Pb concentrations were significantly higher ($P < 0.05$) in sludge-exposed group than in control group (Table 12-22). No differences were apparent in blood levels of other elements in cows.

DISCUSSION

The results of this investigation did not demonstrate risk of increased animal infections after they were grazed on pastures where 2 to 10 dry metric tons/per hectare of sewage sludge had been applied.

The study also indicated that quantitative evaluation of the magnitude of exposure to heavy metals using fecal concentrations in grazing animals should be undertaken soon after the initial 30-day withholding period and one week of grazing on sludge applied pasture. As demonstrated in this study, 3 to 8 months following grazing, fecal Cd levels returned to pre-sludge values. This may be due to washing away of sludge adherent to forage, depletion of sludge-contaminated forage late in grazing, reduced upper soil cadmium concentrations resulting from leaching into the soil bed, uptake by plants and grazing animals, and surface drainage. Uptake, by plants, of heavy metals present in sludge applied to cropland has been demonstrated by several investigators (Edds and Davidson, 1981; Giordano and Mays, 1977; Hinesly *et al.*, 1976, 1977; Miller and Boswell, 1981). Bertrand *et al.*, (1981) allowed beef steers to graze bahiagrass pastures applied with liquid digested sludge, equivalent to 16-32 tons per hectare dry sludge, for 168 days. Several heavy metal concentrations were increased in feces collected at 28 day intervals during grazing. Differences between this study and our results from fecal samples collected at annual herd testing and slaughter can be explained by repeated and higher rates of sludge application, and lack of withholding period before grazing by Bertrand *et al.* (1981) compared to a single low level application and a 30 day withholding period for grazing in our studies. The cattle were allowed to graze on the pastures starting 30 days after sludge application. These results support the recommended 30-days withholding time period in the *Criteria for Classification of Solid Waste Disposal Facilities and Practices* USEPA (1979). An additional contributing factor could be the time lapse between the last grazing and sample collection.

No changes in the blood levels of the elements studied were observed in calves. However, sludge-exposed cows had significantly higher Pb levels compared to control cows. Although this was associated with a tendency for higher Pb levels in feces, liver, and kidneys of cows as well as liver and blood of calves, and also significantly higher Pb levels in calf kidneys, the results should be evaluated cautiously in view of the small sample size: only two cows representing a single farm from the control group (*vs.* 8 animals from 3 farms from the sludge group).

The levels of the elements in various tissues reported here in cattle agree well with those already published (Dorn *et al.*, 1972; Kreuzer, *et al.*, 1976). Among the various tissues studied, kidneys appear to be the most sensitive indicators of long-term low level exposure to heavy metal contaminants. Significant accumulations of Cd and Pb in kidney of calves in this study were associated with a consistent tedency of accumulations of Cd and Pb not only in sludge-exposed calf liver but also in liver and kidneys of older cows on sludge applied farms. A tendency for lower levels of Cu in the liver of both cows and calves in sludge applied farms compared to controls reflects a possible Cd or Pb interaction with Cu. No such interaction was seen in kidney. Similar results were obtained by Lamphere *et al.* (1984) in calves fed normal Cu but elevated (50 ppm) dietary Cd levels. Dorn *et al.* (1973) also reported a very low liver copper level in a cow exposed to Pb contamination from a smelter. Bertrand *et al.* (1981) obtained similar results with beef steers grazing sludge-applied pastures. Cadmium and Zn can directly inhibit Cu uptake by intestinal cells as well as cause Cu entrapment in intestinal cells by intestinal cell metallothionein induction (Mills, 1980). Cadmium can inhibit Cu transport from intestinal cells to the liver by decreasing levels of serum cerruloplasmin (Campbell and Mills (1974). Known reductions in liver Cu concentrations with age (NAS, 1980) are also demonstrated in our study in both sludge-exposed as well as control cattle.

Bone and muscle of either the calves or the cows gave no evidence of accumulation of the elements studied. Hair, on the other hand, exhibited post-sludge increase only in Zn concentration that was also significantly higher than hair Zn concentrations of either pre- or post-sludge control samples. These hair levels, in the absence of such increases in blood and other tissues may suggest direct exposure of hair to sludge-borne Zn and inability of our hair washing procedures to remove adsorbed Zn. The Zn levels in the Columbus sludge (Franklin and Pickaway Counties) was far higher than those levels for the other sewage treatment plants in the health study (Section 1). This hypothesis of an exogeneous source of the Zn is supported by a recent study by Lamphere (1981) in which no demonstrable increase in hair Zn concentration was seen in calves fed Zn levels as high as 600/ug/g of feed for 60 days, in the absence of external contamination. At these levels of feeding, high levels of Zn in blood, liver and kidney were found (Lamphere _et al._, 1984) in association with higher levels of Zn in feces.

The results of our study indicated that land application of sewage-sludge at rates sufficient to supply phosphorous requirements of the soil-crop system (2-10 dry metric tons per hectare) would probably not be associated with increased risk of animal infections with common bacterial and parasitic agents. Evidence of increased exposure to heavy metals obtained during the early grazing period by way of fecal Cd concentrations, disappeared within 4 to 9 months after sludge application and grazing. Heavy metals, especially Cd and Pb, accumulated significantly in calf kidneys following grazing of sludge-applied pastures for a period of 3-8 months beginning one month after sludge application. It is possible that the increases in Cd due to recent sludge-exposure were not significant, due to the inherently (age related) higher levels of Cd in cow kidneys.

The biological significance of Cd and Pb accumulations in calves, demonstrated in this study, is minimized by the low magnitude of accumulations as well as lack of accumulation in muscle, the predominant edible tissue from beef animals. However, the results also suggest that care must be exercised to prevent higher application rates of sludge with high heavy metal concentrations, especially for longer grazing periods than those utilized in this investigation.

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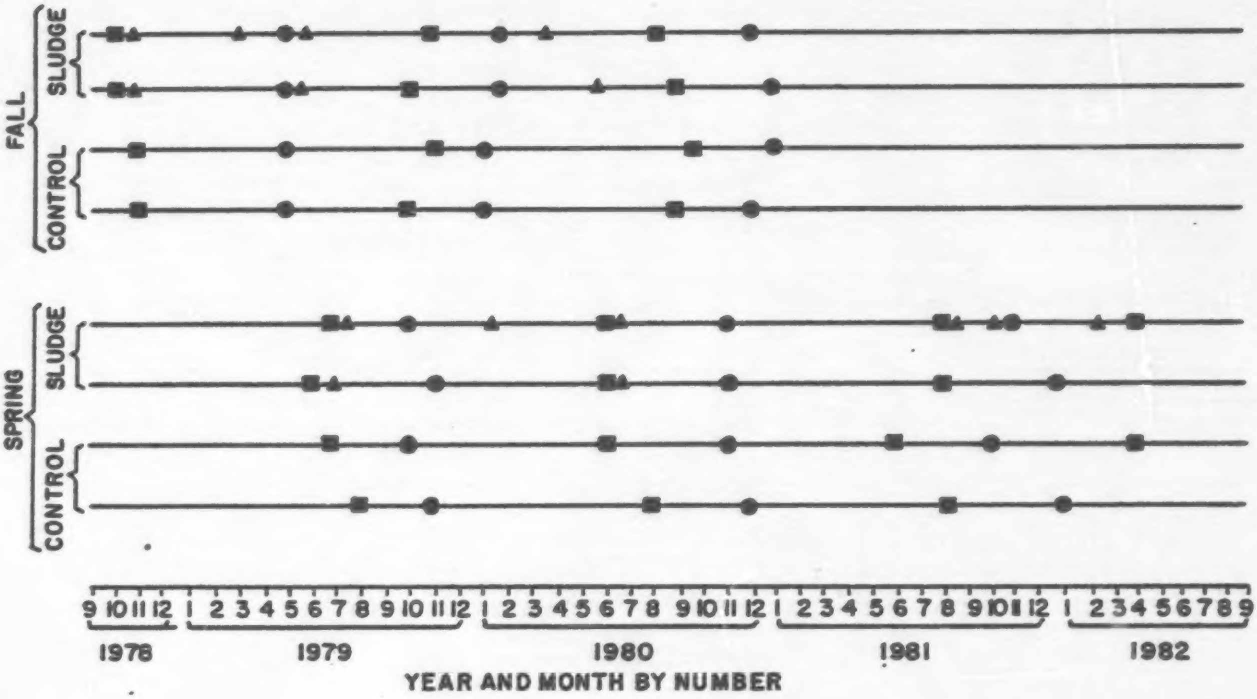

Figure 12-1. Chronology of sludge application (▲), and sample collection during annual herd testing (■), and at slaughter (●), of cattle from sludge and control farms activated in the Fall 1978 or Spring 1979.

TABLE 12-1. ANNUAL CAUDAL FOLD TUBERCULIN TESTING OF CATTLE AND CALVES ON SLUDGE AND CONTROL FARMS

Number of Annual Tests	No. Tested		No. Positive	
	Sludge	Control	Sludge	Control
Total	452	241	0	0
One	334	172	0	0
Two	46	29	0	0
Three	71	37	0	0
Four	1	3	0	0

TABLE 12-2. CERVICAL TEST RESPONSE TO BOVINE TUBERCULIN IN CALVES FROM SLUDGE AND CONTROL FARMS

	Sludge Farms			Control Farms		
		No. of responses			No. of responses	
Year	No. Tested	2.75 mm or greater	4.75 mm or greater	No. tested	2.75 mm or greater	4.75 mm or greater
All	40	0	0	40	1	0
1	8[a]	0	0	8[a]	0	0
2	16	0	0	16	0	0
3	16	0	0	16	1[b]	0

[a] Calves from Springtest farms; the 8 calves from fall test sludge farms and the 8 calves from spring test sludge farms were tested by the caudal fold method prior to slaughter instead of by the cervical method, and were negative.

[b] This animal had a 3 mm increase in skin fold thickness at the bovine site and 4 mm increase at the avian site; therefore according to the USDA it would be negative.

TABLE 12-3. SALMONELLA ISOLATIONS IN FECES FROM ANIMALS ON SLUDGE AND CONTROL FARMS BEFORE SLUDGE (BS) AND AFTER SLUDGE (AS) APPLICATION

	Sludge		Control	
	BS	AS	BS	AS
Animal Type	No. Positive/ No. Tested	No. Positive/ No. Tested	No. Positive/ No. Tested	No. Positive/ No. Tested
ALL	0/36	3/110	0/34	6/105
Bovine	0/26	3/74	0/22	3/74
Swine	0/5	0/17	0/2	2/7
Equine	0/0	0/0	0/4	1/13
Caprine	0/1	0/5	0/0	0/0
Canine	0/4	0/9	0/6	0/11
Feline	0/0	0/1	0/0	0/0
Poultry	0/0	0/4	0/0	0/0

TABLE 12-4. SALMONELLA SPP. ISOLATIONS FROM HUMAN AND ANIMAL FECAL SAMPLES ON SLUDGE AND CONTROL FARMS AND THEIR RELATIONSHIP WITH THE CHRONOLOGY OF SALMONELLA SPP. ISOLATIONS FROM SEWAGE SLUDGE

Group, County and Farm No.	Salmonella Serotype Isolated (Date Sample Collected)		
	Human Feces	Animal Feces[a]	Sludge[b]
SLUDGE FARMS			
Medina County			
1009	S. muenchen (12/5/78)		Negative
Franklin/Pickaway Counties			
4004	S. muenchen (7/24/79)		Negative
		S. muenchen (cattle; 8/25/80)	Negative S. St. Paul (4/29/80)
4017	S. enteritides (11/18/81; 12/13/81)		Negative
4018		S. montevideo (cattle; 6/23/80)	Negative
CONTROL FARMS			
Medina County			
1506	S. java (12/11/78)		
Franklin/Pickaway Counties			
3503	S. heidelberg (6/16/80)		
4519		S. montevideo (horse, swine. cow; 10/2/79)	
		S. montevideo (cattle; 8/12/80)	

[a] Animal fecal samples were only examined in Franklin/Pickaway Counties.

[b] Salmonella spp. isolation of weekly composite sludge sample, representing the time period of sludge application on a farm for all sludge applications preceding each human or animal isolation on this farm. Date accompanying the positive isolation is the date of collection of the sample representing the sludge that was applied on the designated farm immediately preceding the isolation.

TABLE 12-5. COMPARISON OF NUMBERS OF SELECTED PARASITE OVA IN FECAL SAMPLES OF CATTLE FROM SLUDGE AND MATCHING CONTROL FARMS AT THE INDICATED INTERVALS, ALL FARMS

Farm and Time Interval	Number of Samples	Parasite egg count[a]					
		Nematodirus	Strongyle	Strongyloides	Trichuris	Coccidia	Ascaris
SLUDGE							
Pre-sludge	103	3 (0-32)	23 (0-208)	11 (0-160)	5 (0-104)	1182 (0-13200)[b]	0
0-1 Year Post Sludge	130	1 (0-50)	23 (0-400)	17 (0-600)	1 (0-80)	137 (0-8496)	3 (0-50)
1-2 Years Post Sludge	75	0	130 (0-1780)	115 (0-1900)	9 (0-300)	109 (0-1300)	0
2-3 Years Post Sludge	40	0	287 (0-1200)	2 (0-400)	16 (0-400)	230 (0-2550)	6 (0-150)
CONTROL							
Pre-sludge	59	2 (0-24)	28 (0-176)	8 (0-192)	3 (0-56)	861 (0-17328)[c]	0
0-1 Year Post Sludge	59	1 (0-8)	16 (0-192)	3 (0-50)	2 (0-88)	15 (0-100)[d]	0
1-2 Years Post Sludge	83	1 (0-50)	87 (0-600)	43 (0-600)	4 (0-150)	266 (0-5450)	2 (0-150)
2-3 Years Post Sludge	28	0	32 (0-200)	48 (0-500)	12 (0-150)	382 (0-2850)	4 (0-50)

[a] Parasite egg count expressed as mean number of eggs per gram of wet feces. Numbers in parenthesis indicate range for the group of samples.

[b,c,d] Include only 49, 44 and 20 samples respectively. The remaining samples in each group were not included for lack of quantitative results.

TABLE 12-6. DETECTION LIMITS OF VARIOUS HEAVY METALS IN EACH SAMPLE TYPE USING ATOMIC ABSORPTION SPECTROPHOTOMETRY

	Metals			
Sample Type	Cd[a]	Cu[b]	Pb[a]	Zn[c]
Cattle Feces	0.07 μg/g	0.7 μg/g	0.003 μg/g	19 μg/g
Liver	0.003 μg/g	0.2 μg/g	0.04 μg/g	2.5 μg/g
Kidney	0.01 μg/g	0.05 μg/g	0.04 μg/g	1.3 μg/g
Triceps	0.0002 μg/g	0.06 μg/g	0.03 μg/g	1.7 μg/g
Rib	0.0042 μg/g	0.07 μg/g	0.1 μg/g	2.0 μg/g
Hair	0.01 μg/g	0.29 μg/g	0.10 μg/g	0.4 μg/g
Blood	0.022 μg/100 ml	2.3 μg/100 ml	0.8 μg/100 ml	2.6 μg/100 ml

[a] All types of samples were analyzed for cadmium and lead using carbon rod atomization.

[b] Analysis of copper was performed using carbon rod atomization for kidney, rib and blood; and flame atomization (air-acetylene) for other sample types.

[c] Zinc in all sample types was analyzed using flame atomization (air-acetylene).

TABLE 12-7. HEAVY METAL CONCENTRATIONS (μg/g) IN FECES OF CALVES FROM SLUDGE AND CONTROL FARMS

	Concentration (μg/g)[a]			
	Sludge		Control[b]	
Element	Pre-Sludge	Post-Sludge	Pre-Sludge	Post-Sludge
Cadmium	0.373	0.458	0.411	0.320
Copper	22.798	22.926	20.720	16.860
Lead	13.262	11.108	10.044	5.849
Zinc	151.888	136.574	139.424[c]	91.990[c]

[a] Each value is a mean for 46 and 44 individual calves representing 4 sludge and 4 control farms respectively over a period of 3 years. Two replicates of each sample were analyzed in duplicate. The duration of grazing on sludge and control pastures ranged from 3 to 8 months before calves were slaughtered.

[b] Control pre-sludge and post-sludge signify approximately equal periods of time as compared to the sludge exposure farms.

[c] Means within the same treatment group with the same superscript are significantly different ($P < 0.01$).

TABLE 12-8. HEAVY METAL CONCENTRATIONS (μg/g) IN FECES OF CULLED COWS FROM SLUDGE AND CONTROL FARMS

Element	Fecal Concentration (μg/g)[a]	
	Sludge	Control
Cadmium	0.316	0.116
Copper	30.956	18.000
Lead	12.600	8.875
Zinc	104.331	72.800

[a] Each value is a mean of either 10 cows or 2 cows from sludge-receiving and control farms, respectively; cows were on these farms approximately 28 months; the sludge farms received at least 2 annual sludge applications.

TABLE 12-9. FECAL CADMIUM CONCENTRATION BETWEEN SLUDGE AND CONTROL CATTLE SAMPLED YEARLY DURING THE PROJECT

Period	Fecal Cadmium Concentration (μg/g)	
	Sludge (n=58)	Control (n=35)
Pre-Sludge	0.303	0.406
1 yr. Post-sludge	0.359	0.357
2 yr. Post-sludge	0.364	0.275

TABLE 12-10. HEAVY METAL CONCENTRATIONS (μg/g) IN FECAL SAMPLES COLLECTED FROM PASTURES WITHIN ONE MONTH AFTER SLUDGE APPLICATION AND IN PRE-SLUDGE FECAL SAMPLES FROM CATTLE ON SLUDGE AND CONTROL FARMS

	Concentration (μg/g)			
	Sludge		Control	
	Pre-sludge	Post-sludge	Pre-sludge	Post-sludge
Cadmium	0.301 (146)	0.900 (30)[b]	0.451 (93)	0.364 (26)[c]
Copper	22.027 (61)	28.368 (30)	21.131 (60)	22.967 (26)
Lead	12.190 (61)	8.958 (30)	9.714 (58)	7.608 (26)
Zinc	114.076 (61)	135.383 (30)	102.541 (59)	91.462 (26)

[a] Numbers in parenthesis indicate the number of samples analyzed. For cadmium analysis pre-sludge samples include all fecal samples collected from cattle at the very first herd testing. For other elements these numbers represent the number of samples randomly selected for comparison with pasture pick-up (post-sludge) samples.

[b] Significantly different ($P < 0.01$) compared to pre-sludge values within the same treatment group.

[c] Significantly different ($P < 0.05$) compared to pre-sludge values within the same treatment group.

TABLE 12-11. HEAVY METAL CONCENTRATIONS (μg/g) IN CALF LIVERS FROM SLUDGE AND CONTROL FARMS

Element	Liver Concentration (μg/g)[a]	
	Sludge	Control
Cadmium	0.107	0.056
Copper	10.183	21.117
Lead	0.517	0.397
Zinc	43.369	46.924

[a] Each value is a mean for 36 individual calves representing 4 farms over a period of 3 years. Two replicates of each sample were analyzed in duplicate. The duration of grazing on sludge and control pastures ranged from 3 to 8 months before calves were slaughtered.

TABLE 12-12. HEAVY METAL CONCENTRATIONS (μg/g) IN COW LIVER FROM SLUDGE AND CONTROL FARMS

Element	Liver Concentration (μg/g)[a]	
	Sludge	Control
Cadmium	0.234	0.264
Copper	4.405	8.925
Lead	0.526	0.364
Zinc	45.480	55.800

[a] Each value is a mean of either 10 cows or 2 cows from sludge-receiving and control farms, respectively; cows were on these farms approximately 28 months; the sludge farms received at least 2 annual sludge applications.

TABLE 12-13. HEAVY METAL CONCENTRATIONS (μg/g) IN CALF KIDNEYS FROM SLUDGE AND CONTROL FARMS

Element	Kidney Concentration (μg/g)[a]	
	Sludge	Control
Cadmium	0.288[b]	0.174[b]
Copper	3.612	3.959
Lead	0.718[b]	0.415[b]
Zinc	22.507	23.011

[a] Each value is a mean for 36 individual calves representing 4 farms over a period of 3 years. Two replicates of each sample were analyzed in duplicate. The duration of grazing on sludge and control pastures ranged from 3 to 8 months before calves were slaughtered.

[b] Values in the same row with the same superscript are significantly different ($P < 0.05$) only when the analysis of variance was performed using individual Log-transformed values.

TABLE 12-14. HEAVY METAL CONCENTRATIONS (μg/g) IN COW KIDNEY FROM SLUDGE AND CONTROL FARMS

Element	Kidney Concentration (μg/g)[a]	
	Sludge	Control
Cadmium	1.275	1.209
Copper	3.719	3.410
Lead	0.549	0.429
Zinc	22.295	19.500

[a] Each value is a mean of either 10 cows or 2 cows from sludge receiving and control farms, respectively; cows were on these farms approximately 28 months; the sludge farms received at least 2 annual sludge applications.

TABLE 12-15. HEAVY METAL CONCENTRATIONS (μg/g) IN CALF MUSCLE FROM SLUDGE AND CONTROL FARMS

Element	Muscle Concentration (μg/g)[a]	
	Sludge	Control
Cadmium	0.002	0.002
Copper	0.913	0.986
Lead	BDL[b]	BDL[b]
Zinc	39.938	38.533

[a] Each value is a mean for 36 individual calves representing 4 farms over a period of 3 years. Two replicates of each sample were analyzed in duplicate. The duration of grazing on sludge and control pastures ranged from 3 to 8 months before calves were slaughtered.

[b] Below detection limit (detection limit 0.032 μg/g)

TABLE 12-16. HEAVY METAL CONCENTRATIONS (μg/g) IN COW MUSCLE FROM SLUDGE AND CONTROL FARMS

Element	Muscle Concentration (μg/g)[a]	
	Sludge	Control
Cadmium	0.001	0.002
Copper	0.818	1.117
Lead	BDL[b]	BDL[b]
Zinc	55.855	50.625

[a] Each value is a mean of either 10 cows or 2 cows from sludge-receiving and control farms, respectively; cows were on these farms approximately 28 months; the sludge farms received at least 2 annual sludge applications.

[b] Below detection limit (Detection limit 0.032 μg/g)

TABLE 12-17. HEAVY METAL CONCENTRATIONS (μg/g) IN CALF BONE FROM SLUDGE AND CONTROL FARMS

Element	Concentration (μg/g)[a]	
	Sludge	Control
Cadmium	0.038	0.032
Copper	0.234	0.277
Lead	1.402	1.050
Zinc	53.724	59.417

[a] Each value is a mean for 36 individual calves representing 4 farms over a period of 3 years. Two replicates of each sample were analyzed in duplicate. The duration of grazing on sludge and control pastures ranged from 3 to 8 months before calves were slaughtered.

TABLE 12-18. HEAVY METAL CONCENTRATIONS (μg/g) IN COW BONE FROM SLUDGE AND CONTROL FARMS

Element	Concentration (μg/g)[a]	
	Sludge	Control
Cadmium	0.060	0.043
Copper	0.219	0.520
Lead	2.776	3.179
Zinc	63.110	64.550

[a] Each value is a mean of either 10 cows or 2 cows from sludge-receiving and control farms respectively; cows were on these farms approximately 28 months; the sludge farms received at least 2 annual sludge applications.

TABLE 12-19. HEAVY METAL CONCENTRATIONS ($\mu g/g$) IN CALF HAIR FROM SLUDGE AND CONTROL FARMS BY PERIOD IN RESPECT TO SLUDGE EXPOSURE

Element	Hair Concentration ($\mu g/g$)[a]			
	Sludge		Control[b]	
	Pre-sludge	Post Sludge	Pre-sludge	Post Sludge
Cadmium	0.129^c	0.094^c	0.098^c	0.089^c
Copper	6.183^d	4.483^d	6.716^c	5.781^c
Lead	1.116	0.959	1.222	0.912
Zinc	122.705^d	145.136^d	121.879	126.854

[a] Each value is a mean for 48 individual calves representing 4 farms over a period of 3 years. Two replicates of each sample were analyzed in duplicate. The duration of grazing on sludge and control pastures ranged from 3 to 8 months before calves were slaughtered.

[b] Control pre-sludge and post sludge signify approximately equal periods of time as compared to the sludge exposure farms.

[c] Means within the same treatment group for the same element with same superscripts are different ($P< 0.05$).

[d] Means within the same treatment group for the same element with same superscripts are different ($P< 0.01$).

TABLE 12-20. HEAVY METAL CONCENTRATIONS ($\mu g/g$) IN COW HAIR FROM SLUDGE AND CONTROL FARMS

Element	Hair Concentration ($\mu g/g$)[a]	
	Sludge	Control
Cadmium	0.112	0.174
Copper	3.260	5.198
Lead	1.360	2.450
Zinc	134.950	113.000

[a] Each value is a mean of either 10 cows or 2 cows from sludge receiving and control farms, respectively; cows were on these farms approximately 28 months.

TABLE 12-21. HEAVY METAL CONCENTRATIONS (μg/100 ml) IN CALF BLOOD FROM SLUDGE AND CONTROL FARMS

	Concentration (μg/100 ml)[a]			
	Sludge		Control[b]	
	Pre-Sludge	Post-Sludge	Pre-Sludge	Post-Sludge
Cadmium	0.013	0.014	0.013	0.013
Copper	74.302	75.844	87.574	83.149
Lead	9.731	10.956	11.108	9.376
Zinc	305.167	292.144	302.702	297.968

[a] Each value is a mean for 48 and 47 individual calves representing 4 sludge and 4 control farms respectively over a period of 3 years. Two replicates of each sample were analyzed in duplicate. The duration of grazing on sludge and control pastures ranged from 3 to 8 months before calves were slaughtered.

[b] Control pre-sludge and post-sludge signify approximately equal periods of time as compared to the sludge exposure farms.

TABLE 12-22. HEAVY METAL CONCENTRATIONS (μg/100 ml) IN COW BLOOD FROM SLUDGE AND CONTROL FARMS

Element	Concentration (μg/100 ml)[a]	
	Sludge	Control
Cadmium	0.015	0.011
Copper	61.167	69.500
Lead	12.103[b]	4.322[b]
Zinc	299.444	284.750

[a] Each value is a mean of either 9 cows or 2 cows from a sludge-receiving and control farms, respectively; cows were on these farms approximately 28 months; the sludge farms received at least 2 annual sludge applications.

[b] Values with the same superscript are significantly different ($P < 0.05$) by the analysis of variance of log transformed data.

SECTION 13

ESTIMATION OF CADMIUM INTAKE USING FECAL CADMIUM CONCENTRATIONS

Chada S. Reddy, B.V.Sc., M.S., Ph.D.
C. Richard Dorn, D.V.M., M.P.H.
Department of Veterinary Preventive Medicine
College of Veterinary Medicine
The Ohio State University
Columbus, Ohio 43210

ABSTRACT

Increasing use of sludge on private farmlands calls for investigations into the possible translocation of toxic heavy metals such as cadmium (Cd) into the human food chain. Although the use of fecal Cd excretion to estimate daily Cd intake has been suggested and attempted, reliable data on the actual 24 hr. fecal weights are not available. This study was designed to collect 24 hr. fecal samples at 4 month intervals from two groups of farm residents in Ohio. One group of residents lived on farms in which the croplands and/or pastures were amended with 2-10 dry m. tons/hectare of anaerobically digested sewage sludge. The other group served as controls. The fecal samples were analyzed for Cd using atomic absorption spectrophotometer equipped with carbon rod atomizer. The data obtained from this study was used to calculate the daily Cd intakes following correction for absorption (4.6%) in the gastrointestinal tract. Cattle grazing on sludge-amended pastures were also similarly treated except that published 24 hr. fecal weights and a 2% absorption factor was used. The results of this study indicated that the actual 24 hr. fecal weights in different age and sex groups were similar to the extrapolated values based on the National Research Council (NRC) recommended daily caloric requirements. Fecal weights, and thus the daily Cd intakes calculated from these data, although showing no specific age pattern, were significantly higher in males than in females with a female/male ratio of 0.77:1. Daily Cd intakes calculated from these data ranged from 8.87 to 18.52 μg/day for males and 5.37 to 13.31 μg/day for females. Although daily Cd intake for smokers was 1 μg/day higher than non-smokers, the difference was not statistically significant. No significant increase in daily Cd intakes resulted from exposure to sludge on the farmlands. Animals grazing on sludge-amended pastures consumed up to 3 times the amount of Cd consumed by cattle on non-sludge-amended pastures. It was concluded that application of sewage sludge on farmlands at rates of 2-10 dry m. tons/hectare failed to contribute to the daily Cd intake in humans; however, cattle on such farms can increase their Cd consumption significantly.

INTRODUCTION

Among several important toxic heavy metals, cadmium (Cd) is the most widespread contaminant in foods (Mahaffey et al., 1975). Food is by far the major source of Cd to animals and most non-smoking humans (Underwood, 1979). An early estimate of daily average dietary intake of Cd in humans in the U.S. was 50 µg with a range from 4 µg to 92 µg (Friberg et al., 1974). Other studies, however, suggest lower Cd intakes: 30-47 µg/day (Tipton et al., 1969), 26-61 µg/day (Food and Drug Administration, 1977) and 13-16 µg/day (Kowal et al., 1979). Some of these estimates are close to the tolerable dietary level of 57-71 µg/day established by an FAO/WHO expert committee on food additives (1973). Naylor and Loehr (1981) arrived at a maximum safe dietary level of 75 µg/day of Cd after correcting for smoking and allowing for a margin of safety. These data suggest that additional Cd intakes must be avoided in order to prevent the U.S. population from exceeding this daily intake level.

Passage of Federal Water Pollution Control Act of 1972 and the Clean Water Act of 1977 (prohibiting ocean dumping of sewage sludge) has led to a search for alternate methods of disposal of increasing quantities of sewage sludge being produced at the present time. The goals of current sludge management, safe disposal of sewage sludge and effective utilization of available resources, can be achieved by a program involving carefully monitored sludge application on private farm lands (Pahren, 1980).

Sludges, however, especially those derived from waste water contaminated with industrial wastes, can be a significant source of Cd (Chaney, 1980). Uptake of Cd by garden crops grown on sludge-amended soils, increased fecal Cd concentrations in cattle grazing on sludge-applied pastures (Bertrand et al., 1981). Accumulation of Cd in food animal tissues, especially livers and kidneys has also been demonstrated (Fitzgerald, 1980). Recently, Dorn et al. (Section 11) demonstrated that application of treated municipal sewage sludge at rates of 2-10 dry tons/hectare did not produce any observable health effects in human and animal populations on these farms compared to those on a group of control farms from the same geographic areas. Reddy et al. (Section 12), however, showed that even these low application rates can lead to significantly higher Cd accumulations in kidneys of calves grazing sludge-applied pastures for a period of 3 to 8 months. Increase in exposure to Cd was reflected in higher fecal Cd concentrations in the animals during the grazing period (Reddy et al., Section 12). Sludge-borne Cd can find its way to humans by inhalation of dust while working in the fields, consumption of home-produced garden crops, fruits and meat and meat products. Although these possibilities have been pointed out (Ryan et al., 1982) no systematic efforts to evaluate the extent of contribution by sludge to the total Cd intake in humans have been made.

Two methods of direct measurement of total daily Cd intake in humans are: the market basket technique and the total diet collection technique. The market basket technique, involving measurement of Cd concentration of various food stuffs followed by a survey of quantitative consumption of each food ingredient, has yielded daily average Cd intakes of 26-61 µg/day (FDA, 1977).

The total diet collection method, involving collection of a daily sample of each ingredient consumed followed by Cd analysis of the combined homogenate of these samples, has generally yielded lower values than the market basket surveys but these values were closer to the indirect fecal analysis method (Kjellstrom, 1979). Estimation of daily Cd intake has been attempted by the analysis of Cd concentration in fecal samples, multiplication by the amount of daily fecal outputs, and adjustment for the absorption in the gastrointestinal tract. Such a study was initiated by Tipton _et al._, (1969) which resulted in daily Cd intake of 30-47 µg. Recent estimates of daily Cd intake by this method in the U.S. male teenagers are 18 µg in Dallas (Kjellstrom, 1979) and 21 µg in Chicago (Kowal _et al._, 1979). The direct methods suffer from several disadvantages. Food samples in the market basket survey are generally near the limit of detection (Mahaffey _et al._, 1975). Samples collected in the total diet collection method may fail to represent pockets of contamination. Finally, they involve elaborate sample and/or data collection and analysis. Cadmium concentration in feces is reportedly much higher than that in food, which enables more accurate analysis using smaller samples (Iwao _et al._, 1981). Fecal method involves analysis of a single sample material. The fact that only about 4.6 ± 4% of the ingested Cd is absorbed (McLellan _et al._, 1978) will facilitate the correction of total fecal cadmium content by this factor to arrive at total cadmium intake via the alimentary canal. Similarly, it is generally agreed that the fraction of ingested Cd absorbed in animals is about 2% (Friberg _et al._, 1974), thus making it feasible to calculate cadmium intakes for cattle population. Although, the contribution of the biliary excretion and intestinal epithelial secretion and debris to the total fecal Cd is unknown, it will most likely be directly correlated to the total Cd body burden and Cd intake in the days immediately preceding the day of sample collection.

Cigarette smoking can be a significant contributing factor to Cd exposure, especially in heavy smokers (Friberg _et al._ 1974). Up to 2 µg of Cd can be present in each U.S. made cigarette (Menden _et al._, 1972). For each 20 cigarettes smoked 2 to 5 µg of Cd would be inhaled resulting in absorption and retention of as much as 1.2 to 3 µg of Cd (Friberg _et al._, 1974). Although the fraction of Cd in cigarettes that could be excreted in feces has not been estimated, Kjellstrom _et al._ (1978) reported significantly higher fecal Cd excretion rates in a group of smokers _vs._ non-smokers in Stockholm, Sweden. Kowal _et al._ (1979), failed to confirm this effect of smoking on fecal Cd excretions in individuals living in Dallas and Chicago, although the Cd concentrations in urine, blood and tissue samples from smokers among these individuals were significantly higher than those of non-smokers. Also, no consistent trend was observed by Kjellstrom _et al._ (1979). Smokers in Chicago tended to excrete smaller amounts of Cd than non-smokers, whereas the smokers in Dallas tended to excrete higher amounts of Cd than non-smokers. This may be due to the variability in the type of diet consumed and the number of cigarettes smoked. Kowal _et al._ (1979) recognized that their use of estimated fecal weights, based upon extrapolation using the recommended daily caloric intake rather than the actual 24-hr fecal weights, may introduce an error. To date, no comprehensive data on the daily fecal amounts in populations of different age groups is available.

This study presents data on the actual 24-hr fecal weights in individuals of different age groups from Ohio farms. Using Cd concentrations in fecal samples from residents on control and sludge-receiving farms in three different Ohio locations, Cd intakes were calculated for these populations. The possible effect of sludge exposure and smoking in increasing daily Cd intakes in these populations was evaluated. Finally, Cd intakes and the relative contribution of sludge to the daily intake in cattle grazing on sludge-amended pastures was calculated using a 2% correction factor for absorption.

METHODS

The recruitment of farms, their allocation to either the sludge-receiving group or control group, collection data questionnaire, baseline testing and sample collection are described in Section 11 of this report. The criteria for the selection of the eight cattle farms, their random assignment to either the sludge-receiving group or control group and baseline animal data collection are summarized in Section 12. The summary of sludge application rates on each of the farms assigned to receive sludge is presented in Section 1.

Before the first sludge application, residents on each sludge-receiving farm and its matched control farm were asked the following questions to determine their smoking status.

Have you ever smoked: Yes______No______
Do you now smoke? Yes______No______
If yes:
Cigars? Yes______No______
If yes: Average number per week ________________.
Cigarettes? Yes______No______
If yes:. Average number per day______________.
Filtered? Yes______No______
Other? Yes______No______ Specify:__________________________
If you stopped smoking cigarettes, how long ago did you stop?____________

Because of the higher levels of Cd in some garden crops such as lettuce (Friberg et al., 1974), the participants on sludge-receiving farms were instructed not to apply sludge to their gardens. However, deposition of Cd laden dust and uptake of Cd from ground water draining sludge-amended soil can be expected to increase Cd content of garden crops. Edible tissues of cattle grazing sludge-treated pastures would be expected to have higher Cd levels (Kienholz et al., 1976). Therefore, it was decided to look at the extent of consumption of home raised meats and garden crops and their relationship to daily Cd intakes in individuals from sludge-receiving and control farms. The following questionnaire was designed to quantitate such exposures.

What percentage of the meat products in your diet over the past _____ weeks were home-produced? _______%.

What percentage of the garden crops in your diet over the past _____ weeks were home-produced?_______%.

Smoking status and the percentage of diet represented by home-produced meats and garden crops were monitored throughout the period following sludge application by a monthly telephone interview of each participant.

Following sludge application, fecal samples were collected from the participants at 4 month intervals for a period ranging from 6 months to 3 years. Each participant was given a paper bag containing a disposable acid rinsed plastic collection cup (3984, Tupperware, Inc., Hemingway, SC). The participants were asked to collect the total amount of each defecation directly into the cup while sitting normally on the toilet. The collection cup and contents were then placed in another container for transport to the laboratory. Since most westerners normally only have one stool per day (Kjellstrom et al., 1978), these samples were considered to represent a 24 hr. sample. Fecal samples from all the cattle on each of the eight farms chosen to participate in this study were collected into a plastic sleeve and glove (Vet-R-Sem, Loveland, CO) from the rectum before sludge application on sludge-receiving farms. Following application of 2-10 m. tons/hectare of anaerobically digested sewage sludge on the pasture lands, the cattle were kept from grazing for a period of 30 days. At the end of this withholding period, cattle were allowed to graze sludge applied or control pastures. Within one to two weeks from the beginning of grazing, fecal samples representing the herd were picked up from these pastures and placed in paper cups (Dixie, 5 oz., No. 315, Greenwich, CN). Only large piles of fresh individual fecal masses (likely representing excreta from grazing cows as opposed to calves) were sampled.

Fecal Cd analysis. Soon after their arrival into the laboratory, the entire fecal samples were weighed and representative aliquots was transfered to pre-weighed aluminum boats for drying at 80°C to a constant weight. The dry matter was calculated as a percentage of wet weight. The samples were then ground to a uniform powder in a mortar and pestle and stored in 10% nitric acid (HNO_3) washed polyethylene bottles until analyzed for Cd.

Duplicate 0.2 g samples of powdered feces were weighed into clean acid washed 50 ml Kjeldahl flasks. They were digested in 5 ml of conc. HNO_3 (G. Frederick Smith, redistilled, Columbus, Ohio) at a block temperature of 120°C for at least 24 hr. after which 2 X 0.5 ml of 30% hydrogen peroxide (Mallinkrodt, Paris, Kentucky) was slowly added while the flasks were cooling. After the samples stopped bubbling, the flasks were reheated overnight. The acid was then evaporated (to almost complete dryness) taking care to prevent sample combustion. Sample digestates from the Kjeldahl flasks were recovered by the addition of several small volumes of deionized double distilled water along the sides of the neck, thorough swirling of the flask, collecting the washings into a 50 ml volumetric flask and finally making up the volume and storing in polyethylene bottles for analysis. A series of fecal samples with 0, 50, 100 and 150 percent of expected cadmium added (Standard addition series) were also similarly processed for each batch of processed fecal samples. Cadmium analysis was performed using a Varian AA775 series atomic absorption spectrophotometer with background corrector equipped with CRA90 carbon rod atomizer (CRA), ASD53 automatic sample dispenser and DP38 digital printer. Parameters used were: resonance line, 228.8 mm; slit width, 0.5 mm; sample size, 5 ul; dry temp., 90°C; ash temp., 400°C; and atomize temp., 1900°C. All samples with a standard deviation of more than 10% of the average value as well as those with absorbance values higher than the highest standard were appropriately repeated. Detection limit for cadmium by this method was 0.111 μg/g of dry feces. Instrument performance and accuracy of the analyses was confirmed by analyzing a standard set of EPA supplied water samples and comparing the results to those obtained by EPA.

Duplicate 0.2 g samples of dried cattle feces in 30 ml Kjeldahl flasks were added with 10 ml of concentrated HNO_3. After 24 hr of digestion, 0.5 ml of H_2O_2 was added to each sample and they are reheated for an additional 24 hr period. Acid was evaporated to near dryness and the residue dissolved in 25 ml of deionized water. Cadmium was analyzed using CRA as described above. The detection limit was 0.07 μg/g of dry feces.

All reusable glassware and plasticware used in sample analysis were washed thoroughly with a detergent (Event, Economics Laboratory, Inc., St. Paul, Minnesota) in tap water. Cleaned glass or plasticware were rinsed in deionized water and soaked in HNO_3 (1:1) overnight or 4-6 hrs. respectively. The items were then thoroughly rinsed with distilled deionized water and glassware were dried in an oven. The acid rinsings (0.1N HNO_3) from selected items, including sample collection containers, was periodically tested for Cd contamination.

Estimation of Ingested Cd. The procedures of Friberg *et al.*, 1974 and Kjellstrom *et al.*, 1978 were followed. To estimate the daily Cd intake for each age-sex class of participants. The inividual dry fecal weights were multiplied by individual fecal Cd concentrtions and divided by a factor of 0.954 to correct for 4.6% absorption of Cd in the gut.

The formula for daily Cd intake is:

$$\frac{\text{mean fecal Cd concentration } (\mu g/g) \times \text{ mean dry fecal weight } (g/day)}{\text{absorption correction factor, } 0.954}$$

To calculate Cd intakes for cows grazing on sludge-amended pastures, total daily fecal excretion (7.3 lb/day) for adult cows (Cooperative Extension Service-Ohio State University guide, 1980) was multiplied by individual fecal concentrations and divided by 0.98 to correct for absorption (Friberg *et al.*, 1974). The formula

$$\frac{\text{mean fecal Cd concentration } (\mu g/g) \times 3314.2 \text{ g/day}}{\text{absorption correction factor, } 0.98}$$

was used.

Statistical Analysis. The cadmium concentrations were analyzed using arithmetic means. Log transformation of the data resulted in distributions more resembling a normal distribution than the non-transformed data due to the presence of some skewness due to occasional extreme values. After correcting for the error due to sex, smoking status, age group and various interactions, the means for sludge and control groups were tested for differences using heirarchal design of the analysis of variance technique where the error term was based upon farms nested within group differences at the 5% ($P < 0.05$) level. Data for other parameters including fecal weights (wet and dry) and daily Cd intakes were similarly analyzed.

RESULTS

Humans. A total of 122 participants in the sludge group and 103 participants in the control group contributed fecal samples during the project period. Varying lengths of participation by different farm families in the project, as described in Section 11, resulted in individuals contributing 2 to 12 samples. The wet fecal weights ranged from 10.5 g to 285.95 g.

A comparison of average daily dry fecal weights for participants belonging to different age groups (10 year age groups) with those of estimated dry weights (data from Kowal et al., 1979, based upon daily caloric requirements recommended by NRC, multiplied by dry matter content of feces for different age groups from our data) is presented in Tables 13-1 - 13-4. In general, observed fecal dry weights tended to be lower than those estimated as above, especially at the lower-age bracket of 0-9 years. At higher age brackets, the observed values more nearly agreed with estimated values.

Dry fecal weights for all age groups tended to be lower for participants from sludge receiving farms compared to those from control farms. A similar trend was reflected in the total mean daily Cd intake (Table 13-1) for participants from all counties as well as individual counties (13-2 - 13-4). Mean daily Cd intakes estimated as described in Methods for various age groups ranged from 8.87 to 18.52 μg/day in males and 5.37 to 13.31 μg/day in females. The range of individual cadmium intakes (not shown) were 3.01 to 36.04, and 1.99 to 34.74 μg/day, for participants from sludge-receiving farms and control farms, respectively. In general, females tended to have lower dry fecal weights and thus lower daily cadmium intakes.

Statistical analysis of wet and dry fecal weights and daily Cd intake using broader age classifications (under 12, 13-21, 22-59 and 60 and over) among both sludge and control groups indicated no age-related differences in the full general linear model ANOVA (Table 13-5). Also, a comparison between sludge and control group participants (treatment effect) revealed no significant differences. Sex-related differences, however, were noted. Although, all of the parameters analyzed tended to be lower in females compared to males in every age group, significant differences were noted for 22-59 yr. age group as well as for all age groups combined ($P < 0.05$). Daily Cd intakes in females were 3 to 5 μg lower than in males. Participants in Medina and Pickaway and Franklin counties exhibited this trend and contributed to the overall significance. Clark county population, however, failed to show such differences (Tables 13-6 - 13-8).

Mean and peak fecal Cd concentrations, wet and dry fecal weights and daily Cd intake for smokers and non-smokers among individuals from sludge-receiving and control farms are presented in Tables 13-9 - 13-12, for all counties combined and for individual counties. To increase the chances of detecting differences, only individuals that were smoking cigarettes at the beginning of the project and continued to smoke through the sample collection were considered smokers. Non-smokers were defined as those who never smoked cigarettes or any tobacco product and those that were not chewing tobacco during their participation. Peak fecal Cd concentration in smokers from sludge-receiving farms in Medina county was significantly higher ($P \quad 0.05$) compared to the smokers on control farms. The full-model ANOVA, however, failed to show significant smoking effect in any of the parameters studied. There was a consistent trend for higher fecal Cd concentrations in sludge-exposed participants in each of the counties among both smokers and non-smokers. In all counties combined, wet and dry fecal weights as well as daily Cd intakes tended to be lower in participants from sludge-receiving farms compared to those from the control farms. All of these parameters tended to be higher for smokers compared to non-smokers (Tables 13-9 - 13-12). In both sludge and control groups, apparent increase in daily Cd intake due to smoking was about 1 μg.

To evaluate the role of off-farm work in contributing to lower fecal weights in the sludge-exposed group, the data was analyzed by the general linear models ANOVA excluding all participants reporting off-farm work at any time during the project. Although it seemed to have reduced the magnitude of differences between the two groups (data not shown), the participants in the sludge group continued to have a tendency for lower fecal weights. A second possibility was that the participants in the sludge-exposed group could have conscientiously given samples on occasions that they would have postponed, if they were controls. Analysis of the data indicated that there are disproportionately larger numbers of small size samples (Mean - 1 S.D.) in the sludge group compared tb control participants ($P < 0.05$). There were 28 people contributing one or more small size samples in the sludge group compared to 21 in the control group. Among these, 8 participants from the sludge group contributed greater than 50% of their samples in small size category compared to 1 from the control group. Removal of these small size samples did result in the fecal weights being more similar in the two groups (data not shown), but it had no effect on the statistical interpretation of the data.

To evaluate the impact of sludge fully, fecal Cd concentrations, and Cd intakes of participants within the sludge group were examined for a relationship with the magnitude of their exposure to sludge. Particpants were categorized into groups with average weekly duration of sludge exposure (working in sludge fields) of 0, 0-10, 11-45, 46-90, and greater than 90 minutes. Data in Table 13-13 indicates a trend for increasing fecal Cd concentrations as well as increased Cd intake with increasing exposure to sludge. Analysis of variance failed to show significant differences among various categories.

Regression analysis of the relationship between consumption of home-produced meats and garden crops, and daily Cd intakes were not statistically significant.

Animals. Data presented in Table 13-14 indicate that cattle grazing sludge-applied pastures have almost 3 times higher fecal Cd concentration than those in samples from the same animals before grazing on sludge-amended soils ($P < 0.01$). Estimation of Cd intake based upon available data on the amount of feces excreted by adult cattle (cow) and correction for absorption (0.98) yield values of 1.02 and 3.04 mg of Cd/day for cattle before and after exposure to 2-10 dry tons/hectare of sludge, respectively. Animals grazing control pastures, on the other hand, had a slightly lower exposure to Cd during the period corresponding to the post-sludge period compared to pre-sludge period.

DISCUSSION

The results of the study indicated no significant contribution by sludge exposure, at rates ranging between 2-10 dry m. tons/hectare, to the daily Cd intake in humans. To the contrary, the Cd intake tended to be lower in sludge-exposed participants, apparently due to the lower wet and dry fecal weights. Further analysis indicated the possibility of a participant bias leading to greater number of small size samples in the sludge group compared to the control group. Although the possibility of sludge being a source of human dietary cadmium exists, the present study did not demonstrate an increase in Cd intake at the sludge application rates used in this investigation.

Age-related patterns in daily Cd intake have been studied by Kowal, et al., (1979), using extrapolation of the NRC recommended daily caloric requirements to arrive at fecal weights for various age groups. Kjellstrom et al., (1978) actually measured the fecal weights on 3 consecutive days in volunteers ranging from 5 to 69 in age. No specific trend in daily Cd intakes or daily fecal Cd amount was observed in either study. Although Kjellstrom et al., (1978) recommended the use of estimated fecal weights, Kowal et al., (1979) point out the possibility of error in using the estimated fecal weights to calculate daily Cd intake. Our data suggests that this may be true, especially in the case of children aged under 10. The fecal weight measurements for other sex and age groups in our study are in a reasonably good agreement with estimated fecal weights.

Sex differences, demonstrated in this study, confirm similar earlier reported differences. Kjellstrom et al., (1978) found a female/male fecal Cd ratio of 0.80:1, whereas Kowal et al., (1979) found a ratio of 0.72:1 and 0.82:1. When combined (both sludge and control groups), the female/male ratio in our study is 0.77:1, lending support to earlier studies.

Although smoking tended to consistently increase fecal Cd concentrations, the daily Cd intake in smokers was approximately 1 μg/day higher than in non-smokers, in our study. This difference was not statistically significant. Kowal et al., (1979) obtained similar results. Kjellstrom et al., (1978), however, reported a significantly higher (3 μg/day) fecal Cd amount in smokers compared to non-smokers. In addition to the increased food intake, demonstrated in the Swedish study (Kjellstrom et al., 1978), the differences in the Cd content of cigarettes between Sweden and U.S., could explain these differences.

Studies reviewed earlier, using various methods to estimate daily Cd intake, suggest a wide range of intakes. The results of our study generally agree with the U.S. data presented by Kjellstrom et al., (1979). The average

Cd intake in selected Ohio farm families appears to be between 8.87 and 18.52 µg/day for males and 5.37 and 13.31 µg/day for females.

Finally, Kienholz et al., (1976) quoted Chaney's data, estimating that 6% of the dry matter consumed by cattle grazing on sludge-applied pastures, may be sludge adhering to the grass even after a number of rainfalls. The results of our study point to the possibility of using fecal Cd amounts in cattle as an index of Cd dose consumed by cattle from sources of contamination such as sludge, as well as the use of farm animals for environmental monitoring to predict possible human hazards such as cadmium.

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TABLE 13-1. ESTIMATION OF MEAN TOTAL CADMIUM INTAKE IN CONTROL AND SLUDGE RECEIVING FARM PARTICIPANTS BY AGE-SEX GROUPS IN ALL COUNTIES[a]

Age Group and Sex	Dry Weight of Feces Excreted, g/Day			Mean Total Daily Cadmium Intake, μg/Day		
		Observed				
	Expected[b]	Sludge (N)[c]	Control (N)[c]	All Participants	Sludge	Control
0 - 9M	22.1	13.0 (4)	- - -	8.87	8.87	- - -
F	22.1	9.2 (3)	10.5 (4)	5.37	4.66	5.90
10 - 19M	34.4	19.9 (4)	23.5 (6)	14.83	16.35	13.82
F	28.0	17.0 (6)	24.1 (3)	12.94	12.16	14.52
20 - 29M	33.5	20.6 (10)	25.6 (4)	13.92	12.84	16.62
F	25.3	16.1 (7)	23.0 (6)	11.50	8.63	14.84
30 - 39M	32.5	19.7 (12)	26.8 (11)	14.59	12.64	16.73
F	24.9	17.8 (11)	23.4 (10)	10.99	9.14	13.03
40 - 49M	32.5	27.1 (10)	24.5 (6)	14.76	14.44	15.29
F	24.9	19.4 (8)	20.7 (3)	11.84	10.82	14.55
50 - 59M	29.2	23.1 (18)	30.8 (13)	15.09	15.04	15.14
F	23.0	19.0 (6)	22.7 (14)	11.68	10.39	12.23
60 - 69M	28.8	26.2 (8)	25.7 (13)	14.35	15.16	13.85
F	22.4	21.9 (6)	25.7 (8)	13.31	12.85	13.65
70 - 79M	- -	26.4 (3)	28.9 (1)	18.52	19.22	16.39
F	- -	17.6 (3)	16.0 (1)	8.17	7.41	10.44
80 & Over M	- -	32.8 (2)	- - -	17.35	17.35	- - -
F	- -	37.8 (1)	- - -	12.45	12.45	- - -

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[a] Mean total daily cadmium intake represents mean of individual total cadmium intake calculated as:

$$\frac{\text{mean fecal cadmium concentration } (\mu g/g) \text{ X mean dry fecal weight, } (g/day)}{\text{absorption correction factor, } 0.954}$$

[b] Expected daily dry fecal weights are extrapolations based on the caloric requirements (NAS-NRC, 1974) for various age and sex categories, using the total daily fecal amount for age groups 20-40 (Pimparkar et al., 1961).

[c] Number in parentheses indicates the number of participants representing each group's mean.

TABLE 13-2. ESTIMATION OF MEAN TOTAL CADMIUM INTAKE IN CONTROL AND SLUDGE RECEIVING FARM PARTICIPANTS BY AGE-SEX GROUPS IN MEDINA COUNTY[a]

Age Group and Sex	Dry Weight of Feces Excreted, g/Day			Mean Total Daily Cadmium Intake, µg/Day		
	Expected[b]	Observed		All Participants	Sludge	Control
		Sludge (N)[c]	Control (N)[c]			
0 - 9M	22.1	16.1 (1)	- - -	10.53	10.53	- - -
F	22.1	11.1 (1)	3.1 (1)	4.62	6.12	3.12
10 - 19M	34.4	- - -	- - -	- - -	- - -	- - -
F	28.0	20.8 (1)	28.3 (1)	13.99	11.11	16.86
20 - 29M	33.5	- - -	- - -	- - -	- - -	- - -
F	25.3	10.1 (1)	17.5 (1)	9.01	8.17	9.85
30 - 39M	32.5	11.9 (2)	29.1 (3)	15.30	9.48	19.19
F	24.9	24.5 (3)	16.9 (2)	14.61	17.48	10.29
40 - 49M	32.5	26.3 (2)	20.8 (1)	12.56	13.65	10.38
F	24.9	24.5 (2)	4.3 (1)	11.87	16.65	2.31
50 - 59M	29.2	19.5 (5)	29.6 (4)	12.00	13.08	10.66
F	23.0	22.6 (3)	20.4 (3)	12.14	12.40	11.88
60 - 69M	28.8	23.8 (3)	28.1 (2)	15.12	15.67	14.31
F	22.4	22.6 (2)	17.4 (1)	8.50	9.35	6.81
70 - 79M	- -	- - -	- - -	- - -	- - -	- - -
F	- -	- - -	- - -	- - -	- - -	- - -
80 & Over M	- -	- - -	- - -	- - -	- - -	- - -
F	- -	- - -	- - -	- - -	- - -	- - -

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[a] Mean total daily cadmium intake represents mean of individual total cadmium intake calculated as:

$$\frac{\text{mean fecal cadmium concentration } (\mu g/g) \times \text{mean dry fecal weight, } (g/day)}{\text{absorption correction factor, } 0.954}$$

[b] Expected daily dry fecal weights are extrapolations based on the caloric requirements (NAS-NRC, 1974) for various age and sex categories, using the total daily fecal amount for age groups 20-40 (Pimparkar *et al.*, 1961).

[c] Number in parentheses indicates the number of participants representing each group's mean.

TABLE 13-3. ESTIMATION OF MEAN TOTAL CADMIUM INTAKE IN CONTROL AND SLUDGE RECEIVING FARM PARTICIPANTS BY AGE-SEX GROUPS IN FRANKLIN-PICKAWAY COUNTY[a]

Age Group and Sex	Dry Weight of Feces Excreted, g/Day			Mean Total Daily Cadmium Intake, μg/Day		
		Observed				
	Expected[b]	Sludge (N)[c]	Control (N)[c]	All Participants	Sludge	Control
0 - 9 M	22.1	12.0 (3)	- - -	8.31	8.31	- - -
F	22.1	8.3 (2)	12.9 (3)	5.67	3.93	6.83
10 - 19 M	34.4	19.9 (4)	24.3 (4)	14.75	16.35	13.15
F	28.0	15.8 (3)	22.1 (2)	12.82	12.47	13.35
20 - 29 M	33.5	20.5 (6)	25.3 (3)	13.32	11.46	17.04
F	25.3	15.1 (4)	26.7 (4)	11.84	6.13	17.56
30 - 39 M	32.5	20.6 (7)	28.8 (5)	15.83	13.68	18.84
F	24.9	16.1 (7)	22.5 (5)	8.05	6.32	10.48
40 - 49 M	32.5	27.3 (8)	25.2 (5)	15.27	14.64	16.27
F	24.9	18.4 (5)	28.8 (2)	12.40	9.09	20.67
50 - 59 M	29.2	26.5 (8)	26.8 (4)	16.04	17.33	13.47
F	23.0	19.0 (2)	22.2 (8)	10.65	10.16	10.78
60 - 69 M	28.8	26.3 (4)	24.4 (8)	14.03	15.69	13.20
F	22.4	21.5 (4)	26.5 (4)	13.87	14.60	13.14
70 - 79 M	- -	26.5 (3)	28.9 (1)	18.52	19.22	16.39
F	- -	21.6 (2)	16.0 (1)	9.77	9.44	10.44
80 & Over M	- -	32.8 (2)	- - -	17.35	17.35	- - -
F	- -	37.9 (1)	- - -	12.45	12.45	- - -

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[a] Mean total daily cadmium intake represents mean of individual total cadmium intake calculated as:

$$\frac{\text{mean fecal cadmium concentration } (\mu g/g) \times \text{ mean dry fecal weight, } (g/day)}{\text{absorption correction factor, } 0.954}$$

[b] Expected daily dry fecal weights are extrapolations based on the caloric requirements (NAS-NRC, 1974) for various age and sex categories, using the total daily fecal amount for age groups 20-40 (Pimparkar *et al.*, 1961).

[c] Number in parentheses indicates the number of participants representing each group's mean.

TABLE 13-4. ESTIMATION OF MEAN TOTAL CADMIUM INTAKE IN CONTROL AND SLUDGE RECEIVING FARM PARTICIPANTS BY AGE-SEX GROUPS IN CLARK COUNTY[a]

Age Group	Dry Weight of Feces Excreted, g/Day			Mean Total Daily Cadmium Intake, μg/Day		
		Observed				
and Sex	Expected[b]	Sludge (N)[c]	Control (N)[c]	All Participants	Sludge	Control
0 - 9M	22.1	- - -	- - -	- - -	- - -	- - -
F	22.1	- - -	- - -	- - -	- - -	- - -
10 - 19M	34.4	- - -	22.0 (2)	15.16	- - -	15.16
F	28.0	16.9 (2)	- - -	12.21	12.21	- - -
20 - 29M	33.5	20.9 (4)	26.7 (1)	14.99	14.90	15.33
F	25.3	21.1 (2)	13.5 (1)	12.23	13.86	9.97
30 - 39M	32.5	23.1 (3)	21.1 (3)	11.53	12.31	10.74
F	24.9	9.7 (1)	29.3 (3)	15.28	3.82	19.19
40 - 49M	32.5	- - -	- - -	- - -	- - -	- - -
F	24.9	14.7 (1)	- - -	7.81	7.81	- - -
50 - 59M	29.2	21.4 (5)	35.0 (5)	7.81	7.81	- - -
F	23.0	8.2 (1)	26.2 (3)	13.53	4.82	16.44
60 - 69M	28.8	33.5 (1)	27.4 (3)	14.32	11.49	15.26
F	22.4	- - -	27.5 (3)	16.60	- - -	16.60
70 - 79M	- -	- - -	- - -	- - -	- - -	- - -
F	- -	9.5 (1)	- - -	3.34	3.34	- - -
80 & Over M	- -	- - -	- - -	- - -	- - -	- - -
F	- -	- - -	- - -	- - -	- - -	- - -

[a] Mean total daily cadmium intake represents mean of individual total cadmium intake calculated as:

$$\frac{\text{mean fecal cadmium concentration } (\mu g/g) \times \text{ mean dry fecal weight, } (g/day)}{\text{absorption correction factor, } 0.954}$$

[b] Expected daily dry fecal weights are extrapolations based on the caloric requirements (NAS-NRC, 1974) for various age and sex categories, using the total daily fecal amount for age groups 20-40 (Pimparkar *et al*., 1961).

[c] Number in parentheses indicates the number of participants representing each group's mean.

TABLE 13-5. DAILY FECAL WEIGHTS AND CADMIUM INTAKE IN SPECIFIC AGE-SEX GROUPS IN SLUDGE-EXPOSED AND CONTROL PARTICIPANTS, ALL COUNTIES

Age Group and Sex	Daily Fecal Weights (g)				Cd intake µg/day	
	Wet[a]		Dry			
	Sludge (N)	Control (N)	Sludge	Control	Sludge	Control
Under 12M	59.0 (7)	86.7 (3)	16.1	25.1	12.55	13.26
F	50.3 (8)	49.6 (6)	14.3	15.2	9.48	8.84
13 - 21M	89.4 (2)	83.4 (3)	23.5	22.0	12.95	14.38
F	35.0 (3)	78.9 (3)	11.6	22.1	7.77	17.56
22 - 59M[b]	97.4 (49)	123.8 (34)[b]	22.5[b]	27.8[b]	13.93[b]	15.85[b]
F[b]	76.8 (30)	87.9 (31)[b]	18.6[b]	22.8[b]	9.92[b]	12.76[b]
60 up M	117.7 (13)	111.2 (14)	27.3	25.9	16.43	14.03
F	104.3 (10)	96.5 (9)	22.2	24.6	11.18	13.29
All Ages M	97.1 (71)[b]	116.2 (54)[b]	22.8[b]	26.8[b]	14.22[b]	15.16[b]
F	75.6 (51)[b]	84.2 (49)[b]	18.2[b]	22.2[b]	9.97[b]	12.67[b]

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[a] (N) represents the number of people contributing one or more fecal samples under each age-sex group and applies to all other parameters for the respective treatment and age-sex group.

[b] Significantly different between males and females within each group ($P < 0.05$).

TABLE 13-6. DAILY FECAL WEIGHTS AND CADMIUM INTAKE IN SPECIFIC AGE-SEX GROUPS IN SLUDGE-EXPOSED AND CONTROL PARTICIPANTS, MEDINA COUNTY

Age Group and Sex	Daily Fecal Weights (g)				Cd intake µg/day	
	Wet[a]		Dry			
	Sludge (N)	Control (N)	Sludge	Control	Sludge	Control
Under 12M	48.3 (1)		16.1		10.53	
F	59.6 (2)	46.1 (2)	16.0	15.7	8.62	9.99
13 - 21M						
F	31.2 (1)		10.1		8.18	
22 - 59M	80.8 (9)	127.8 (8)	19.3	28.3	12.41	13.82
F	88.2 (8)	64.3 (7)	23.8	16.7	15.37	9.77
60 up M	102.5 (3)	108.6 (2)	23.8	28.1	15.67	14.31
F	115.0 (2)	69.4 (1)	22.6	17.4	9.35	6.81
All Ages M	83.3 (13)	123.9 (10)[b]	20.1	28.3	13.01	13.92
F	83.5 (13)	61.2 (10)[b]	21.3	16.6	12.85	9.52

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[a] (N) represents the number of people contributing one or more fecal samples under each age-sex group and applies to all other parameters for the respective treatment and age-sex group.

[b] Significantly different between males and females within each group ($P < 0.05$).

TABLE 13-7. DAILY FECAL WEIGHTS AND CADMIUM INTAKE IN SPECIFIC AGE-SEX GROUPS IN SLUDGE-EXPOSED AND CONTROL PARTICIPANTS, FRANKLIN-PICKAWAY COUNTIES

Age Group and Sex		Daily Fecal Weights (g)				Cd intake µg/day	
		Wet[a]		Dry			
		Sludge (N)	Control (N)	Sludge	Control	Sludge	Control
Under 12	M	60.7 (6)	86.7 (3)	16.1	25.1	12.88	13.26
	F	43.1 (4)	51.4 (4)	12.1	15.0	8.54	8.26
13 - 21	M	89.4 (2)	88.5 (1)	23.5	22.0	12.95	12.81
	F	36.9 (2)	78.9 (3)	12.4	22.1	7.57	17.56
22 - 59	M	108.3 (28)	116.4 (17)	23.9	26.6	14.55	16.50
	F	77.7 (17)	91.9 (17)	17.3	24.2	7.68	12.45
60 up	M	123.4 (9)	106.1 (9)	27.8	24.9	17.24	13.56
	F	110.4 (7)	96.0 (5)	23.9	24.4	12.82	12.60
All Ages	M	104.2 (45)[b]	109.4 (30)[b]	23.6[b]	25.8[b]	14.79[b]	15.17[b]
	F	78.0 (30)[b]	85.7 (29)[b]	17.8[b]	22.7[b]	8.99[b]	12.42[b]

[a] (N) represents the number of people contributing one or more fecal samples under each age-sex group and applies to all other parameters for the respective treatment and age-sex group.

[b] Significantly different between males and females within each group ($P < 0.05$).

TABLE 13-8. DAILY FECAL WEIGHTS AND CADMIUM INTAKE IN SPECIFIC AGE-SEX GROUPS IN SLUDGE-EXPOSED AND CONTROL PARTICIPANTS, CLARK COUNTY

Age Group and Sex		Daily Fecal Weights (g)				Cd intake µg/day	
		Wet[a]		Dry			
		Sludge (N)	Control (N)	Sludge	Control	Sludge	Control
Under 12	M						
	F	55.6 (2)		16.8		12.21	
13 - 21	M		80.9 (1)		22.0		15.16
	F						
22 - 59	M	84.4 (12)	134.3 (9)	21.6	29.4	13.61	16.44
	F	55.5 (5)	101.7 (7)	15.0	25.7	8.83	16.51
60 up	M	112.8 (1)	128.2 (3)	33.4	27.3	11.49	15.26
	F	40.0 (1)	106.4 (3)	9.5	27.5	3.34	16.60
All Ages	M	86.6 (13)	125.4 (13)	22.6	27.9	13.45	16.60
	F	53.6 (8)	103.1 (10)	14.8	26.2	8.99	16.54

[a] (N) represents the number of people contributing one or more fecal samples under each age-sex group and applies to all other parameters for the respective treatment and age-sex group.

TABLE 13-9. FECAL CADMIUM CONCENTRATION, FECAL WEIGHT AND DAILY CADMIUM INTAKE FOR SMOKERS[a] AND NON-SMOKERS[b] ON SLUDGE AND CONTROL FARMS, ALL COUNTIES

Category	Mean Concentration (µg/g)			Fecal Weight (g)		
	No.	Mean[c]	Peak[d]	Wet	Dry	Cd Intake g/day
Sludge						
All Persons	128	0.574	0.865	84.6	20.6	12.20
Smokers	27	0.607	0.870	91.5	21.0	12.99
Non-smokers	101	0.565	0.864	82.7	20.5	11.99
Control						
All Persons	83	0.541	0.783	96.3	24.2	13.67
Smokers	13	0.550	0.816	103.6	23.3	14.49
Non-smokers	70	0.540	0.777	94.9	24.4	13.52

[a] Persons who smoked cigarettes during the entire project period.

[b] Persons who never smoked any tobacco product throughout their life. Persons who chewed tobacco were not included in the analysis.

[c] Mean of all persons' average fecal Cd value during the post-sludge application period.

[d] Mean of all persons' peak fecal Cd value during the post-sludge application period.

TABLE 13-10. FECAL CADMIUM CONCENTRATION, FECAL WEIGHT AND DAILY CADMIUM INTAKE FOR SMOKERS[a] AND NON-SMOKERS[b] ON SLUDGE AND CONTROL FARMS, MEDINA COUNTY

Category	Mean Concentration (µg/g)			Fecal Weight (g)		
	No.	Mean[c]	Peak[d]	Wet	Dry	Cd Intake g/day
Sludge						
All Persons	20	0.609	0.884	80.3	20.7	12.88
Smokers	2	0.690	1.019[e]	51.0	13.9	10.52
Non-smokers	18	0.600	0.869	83.6	21.4	13.14
Control						
All Persons	18	0.521	0.719	77.8	20.2	11.12
Smokers	2	0.456	0.627[e]	62.8	15.5	8.56
Non-smokers	16	0.529	0.731	79.7	20.8	11.44

[a] Persons who smoked cigarettes during the entire project period.

[b] Persons who never smoked any tobacco product throughout their life. Persons who chewed tobacco were not included in the analysis.

[c] Mean of all persons' average fecal Cd value during the post-sludge application period.

[d] Mean of all persons' peak fecal Cd value during the post-sludge application period.

[e] Significantly higher for smokers on sludge receiving farms than on control farms ($P < 0.05$) by the hierarchical design of the ANOVA technique where the error term was based upon differences among farms within county and treatment groups.

TABLE 13-11. FECAL CADMIUM CONCENTRATION, FECAL WEIGHT AND DAILY CADMIUM INTAKE FOR SMOKERS[a] AND NON-SMOKERS[b] ON SLUDGE AND CONTROL FARMS, FRANKLIN AND PICKAWAY COUNTIES

Category	Mean Concentration (µg/g)			Fecal Weight (g)		
	No.	Mean[c]	Peak[d]	Wet	Dry	Cd Intake µg/day
Sludge						
All Persons	79	0.567	0.867	88.6	21.0	12.29
Smokers	21	0.621	0.864	91.3	20.6	13.27
Non-smokers	58	0.547	0.869	87.6	21.1	11.93
Control						
All Persons	47	0.555	0.821	93.0	23.7	13.71
Smokers	8	0.594	0.919	109.7	24.1	16.41
Non-smokers	39	0.547	0.801	89.6	23.7	13.16

[a] Persons who smoked cigarettes during the entire project period.

[b] Persons who never smoked any tobacco product throughout their life. Persons who chewed tobacco were not included in the analysis.

[c] Mean of all persons' average fecal Cd value during the post-sludge application period.

[d] Mean of all persons' peak fecal Cd value during the post-sludge application period.

TABLE 13-12. FECAL CADMIUM CONCENTRATION, FECAL WEIGHT AND DAILY CADMIUM INTAKE FOR SMOKERS[a] AND NON-SMOKERS[b] ON SLUDGE AND CONTROL FARMS, CLARK COUNTY

Category	Mean Concentration (µg/g)			Fecal Weight (g)		
	No.	Mean[c]	Peak[d]	Wet	Dry	Cd Intake µg/day
Sludge						
All Persons	29	0.569	0.847	76.5	19.6	11.50
Smokers	4	0.491	0.829	112.6	26.1	12.80
Non-smokers	25	0.582	0.850	70.7	18.6	11.29
Control						
All Persons	18	0.526	0.748	123.3	29.5	16.12
Smokers	3	0.494	0.669	114.6	26.3	13.33
Non-smokers	15	0.533	0.764	125.1	30.1	16.68

[a] Persons who smoked cigarettes during the entire project period.

[b] Persons who never smoked any tobacco product throughout their life. Persons who chewed tobacco were not included in the analysis.

[c] Mean of all persons' average fecal Cd value during the post-sludge application period.

[d] Mean of all persons' peak fecal Cd value during the post-sludge application period.

TABLE 13-13. RELATIONSHIP BETWEEN SLUDGE-EXPOSURE AND CADMIUM INTAKE IN PARTICIPANTS FROM SLUDGE-RECEIVING FARMS, ALL COUNTIES[a]

Duration of Sludge Exposure (min/wk)	No.	Dry Fecal Weight (g/day)	Cadmium Concentration (μg/g)	Cadmium Intake (μg/day)
0	11	24.4	0.490	11.82
0-10	7	17.9	0.473	8.77
11-45	8	22.0	0.576	12.40
46-90	10	30.7	0.538	16.55
91 up	14	27.3	0.572	15.51

[a] Only those individuals with no off-farm work are included. Samples weighing below one standard deviation of the overall mean are excluded from this data.

TABLE 13-14. ESTIMATION OF CADMIUM INTAKE IN CATTLE GRAZING SLUDGE-AMENDED PASTURES[a]

Farm Group and Period	Fecal Cd Concentration μg/g	Fecal Weight: Solids (kg/day)[b]	Cd Intake[c] (μg/day)
Sludge			
Pre-	0.301[d]	3.314	1017.9[d]
Post-	0.900[d]	3.314	3043.5[d]
Control			
Pre-	0.451	3.314	1525.1
Post-	0.364	3.314	1230.9

[a] Post-sludge samples collected from fresh large fecal masses from pastures being grazed by cattle, presumed to be from older cows.

[b] From "Ohio Livestock Waste Management Guide", Cooperative Extension Service, The Ohio State University, Bulletin No. 604, 1980, p. 14.

[c] Estimated by multiplying Cd concentration with fecal weight and then dividing by correction factor 0.98.

[d] Significantly different from each other within the same treatment group ($P < 0.01$).

OVA AND LARVAE ON PASTURE FORAGE AFTER MUNICIPAL SEWAGE SLUDGE APPLICATION

David N. Lamphere, D.V.M., Ph.D.
William J. Zingalie, D.V.M.
C. Richard Dorn, D.V.M., M.P.H.
Department of Veterinary Preventive Medicine
College of Veterinary Medicine
The Ohio State University
Columbus, Ohio 43210

SUMMARY

Soil and forage samples were collected before sludge application and 7, 14 and 28 days after application in sludge treated and non-treated (control) pasture areas on 3 farms in Pickaway County, Ohio. A standardized sampling pattern was used for both soil and forage samples. Only one parasitic ascarid (Ascaris or Toxocara) ovum was found. It was in a forage sample from a sludge treated area collected on day 14 after sludge application. There were no consistent differences in the concentration of larvae in either soil or forage samples from sludge treated and control areas. There were more free-living larvae identified than parasitic larvae in both treated and control area forage samples. The predominant free-living larvae observed were of the family Rhabditidae. There appeared to be an increase in the numbers of these larvae during a period of increased rainfall and temperature. The risk of transmission of pathogenic parasites via sludge application at a rate of 2-10 dry metric tons per hectare on cattle pasture appears to be minimal.

INTRODUCTION

Various parasitic organisms have been shown to be present in raw sewage and sludges from municipal treatment plants. Of these, Ascaris, Toxascaris, Toxocara, Ancylostoma, Necator, Capillaria, Trichuris, and Taenia species appear to be the most common, and able to withstand sludge treatment process (Hayes, 1977; Little, 1980). Although these organisms appear to exist in treated sludge in small numbers, potential hazards involving parasitism should be evaluated carefully so that persons choosing to apply sludge on farmland will be fully informed. This study was designed to answer the following questions: Is there an increased concentration of ova and larvae on the forage grown on fields where sludge has been applied? Are there differences in the presence of ova and larvae on vegetation following sludge application?

MATERIALS AND METHODS

Procedures for Recovery of Larvae and Ova from Pasture Forage Samples

Three cattle farms with pastures in Pickaway County, Ohio were chosen for this study. Each pasture was divided into a sludge applied (treated) area and a control (not treated) area. Anaerobically digested sludge from the Columbus Sewage Treatment Facility was applied to the treated area at a rate of 2-4 dry metric tons per hectare. Approximately one-half to one acre of the treated area and one-half to one acre of a control area of the pasture were selected for sampling. Beginning in one corner of the sample area, the sampler walked the area in a pattern resembling the letter W according to the method of Taylor (1939). Every five to ten paces, the sampler stopped and collected three samples; one in front, one to the right and one to the left. The sample was collected by grasping a sample of forage as close to the ground as possible, and then breaking it off to avoid pulling up the roots. Manure piles were avoided when collecting the sample.

Once the sample area had been walked in one direction, the pattern was repeated again in the opposite direction. This yielded about one to five pounds of forage per sample.

The forage sample was examined within 24 hours of sampling to avoid deterioration of any existing larvae or ova. The sample was stored in the refrigerator until processing began.

The extraction process was a modification of the method described by Heath and Major (1968). Approximately 450 grams of the forage sample was weighed and placed in a 12 liter bucket. The weighing was accomplished on a triple beam balance and the weight was recorded. The sample was washed manually with a high pressure spray of tap water sufficient to cover the sample in the bucket. After washing, a few grams of commercial laundry detergent was added to the sample and mixed with the water. The sample was then allowed to stand overnight.

The next day, the volume was reduced to about 800 ml by siphoning away the supernatant water. The remaining sediment was collected and divided into four large (230 ml) plastic bottles. The samples were then centrifuged for 15 minutes at 2,000 rpm.

Each bottle was decanted until only 25 ml of sediment was left in the bottle. Each 25 ml of sediment was added to a 50 ml centrifuge tube with 25 ml of saturated sodium nitrate solution (sp. gr. 1.40). Each tube was mixed thoroughly by inversion. The four centrifuge tubes contained a total of 200 ml with all the larvae and ova recovered from the processed pasture sample. The samples were centrifuged for 15 minutes at 1,200 rpm. Approximately 50 ml of supernatant fluid from each of the four tubes was decanted into a graduated cylinder.

For identification of parasitic and free-living larvae, a sample was withdrawn from the collected supernatant fluid (after agitation) using a McMaster straining pipette and one counting chamber (0.3 ml) of a McMaster slide was filled (Dunn, 1978). The slide was examined under a microscope at low magnification for the presence of parasitic ova and larvae. The total volume was then counted in a 20 ml counting chamber.

The supernate (approximately 200 ml) was allowed to stand for 15 minutes. The top 2 ml was then removed with a Pasteur pipette and placed into 18 ml of 10% formalin for storage at 4°C until counting was performed.

The recovered larvae and ova were classified into one of two categories; parasitic or free-living. Differentation of parasitic larvae from free-living larvae was based primarily on the presence of a sheath around the parasitic form. This sheath gave a halo effect and also gave the impression of a sharply tapering tail. In contrast, the free-living nematodes were unsheathed and the tail was more bluntly tapered and rounded.

Further classification of parasite and free-living forms into species was accomplished by a more detailed examination of the mouth parts and the intestinal tracts. The ova were classified into parasitic and free-living forms primarily on structure and morphology.

Procedure for Recovery of Larvae and Ova from Pasture Soil Samples

Top soil samples were collected from a sampling area using a small metal spatula and walking the same "W" pattern previously described for that of forage collection. Approximately 65 to 80 grams of top soil were obtained and thoroughly mixed. Four grams of mixed top soil were weighed out on a triple beam balance and placed on a four ounce paper cup.

The contents of the cup were mixed vigorously with 56 ml of saturated sodium nitrate solution (sp. gr. 1.40). The McMaster straining pipette was used to draw out a quantity of the solution as quickly as possible after mixing. The McMaster counting chamber was filled and the slide was examined under the microscope at 100 X magnification after it had been allowed to stand for three to five minutes. The same basis of classification into parasite and free-living forms was used to record the numbers of ova and larvae in soil samples.

Collection of Weather Data

A Taylor graduated, tapered-cylinder type raingauge was placed on each of the 3 farms. The farmers recorded the rainfall in inches. The investigators periodically confirmed the recordings, and calculated the number of inches of rainfall on each farm for each week of the study.

RESULTS

Microscopic examination of samples revealed only ascarid and Strongyle-type parasitic species, in addition to many free-living species. Before sludge application, there were similar concentrations of total larvae on forage collected from both the area designated for sludge application and for control on all three of the farms (Tables 14-1 - 14-3). After sludge application, the samples from the sludge application and the control areas on a given farm continued to have generally similar concentrations. There was no regular pattern consistent with a sludge effect. In fact, on Farm No. 4017 (Table 14-2) the control samples had higher concentrations of larvae than did the sludge applied samples. On Farm No. 3005 (Table 14-3) the 7 day and the 14 day control samples were higher than the samples from the sludge applied areas but the reverse was observed for the 28 day samples. The numbers of larvae observed per sample ranged from 5 to 129.

The largest increase in larvae concentration was in the 2nd and 3rd weeks in July. This was also the same period when daily temperatures reached the low 90's. The rainfall data also showed that this period had more inches of rain per week than the last part of the month.

The increase in rainfall during the 2nd and 3rd weeks of July was associated with an increase in larvae concentration. Increased rainfall during hot summer months is favorable to bacterial growth, which is the chief food for these nematodes and could account for the increase in larvae concentrations during this time period. Rainfall also could facilitate the escape of larvae from dried, encrusted sludge accumulations (Dunn, 1978).

The results of the larval identification revealed more free-living larvae than parasitic larvae with a ratio of free-living to parasitic larvae of 4.67 to 1 for all samplings combined (Table 14-4). The small numbers of larvae identified in each sample precluded a comparison by length of time after sludge application. Of the free-living nematodes which were recovered, the predominant form was of the family Rhabditidae (Herd et al., 1980).

Only one parasitic ovum was recovered and identified from the processed forage samples. The positive sample was from a sludge treated pasture (Farm No. 4017). The ovum was identified as an ascarid (Ascaris or Toxocara) species.

DISCUSSION

Although free-living nematodes are normally considered harmless, past work performed by Chang et al. (1960) and Chang (1961) indicated that these organisms found in water treatment plants, can ingest pathogens which are then protected from lethal effects of the water disinfection process. The same reasoning can also be applied to those nematodes found on the sludge pastures. There exists the possibility of these nematodes ingesting any pathogens which might be found in the applied sludge, and therefore protecting them from exposure to heat and UV radiation. With an increase in the number of free-living nematodes on the pasture, there may be a concurrent increase in the chance of disease transmission to the grazing cattle.

It is interesting to note that the one parasitic ovum which was recovered was an ascarid ovum. Ascarid ova are more resistant to sewage treatment processes than other parasites (Wright, et al., 1942). This raises the possibility of transmission of these eggs found in sludge to animals having access to the pastures. While it is reasonable to assume that the ascarid egg may have been presented to the pasture via the sludge, one must not overlook the possibility that this egg could have been passed by the owner's dog that often accompanied the owner out to the pasture. Although it has been documented that ova of Ascaris, Toxascaris, Toxocara, Trichuris, Hymenolepsis, and Taenia species can withstand the treatment of raw sewage and therefore be found in sludges in minimal numbers (Hayes, 1977), it is reasonable to assume that these parasites may be missed in sampling when the sludge is spread over a pasture and therefore diluted out.

Of the three farms participating in the study, all of the pastures sampled were composed of a combination of orchard grass, timothy and clover. Two of the pastures had a fairly good growth while the pasture on Farm No. 3005 had been grazed down prior to the application of sludge.

The cattle herds from Farms No. 4017 and No. 4018, were left on the pastures for the period of sample collecting. The herd from Farm No. 4018 seemed to favor the sludge-spread side of the field and spent the majority of its time grazing that half, although it would avoid any large stockpiles of sludge if present.

During the one month of this study, the herd from Farm No. 4017 was never seen on the sludge-spread half of the pasture and there was little evidence it was grazing that half. The herd spent the majority of its time along the banks of the creek which ran across the far end of the field. Although it ran across the pasture, the immediate area around the creek received no sludge due to the difficulty in hauling sludge to the area.

Farm No. 3005 kept the cattle herd off the sludge-spread pasture the entire month of collection primarily to allow the grasses to grow up again. The cattle were not observed after they were returned to the pasture.

The results of this study indicate that the use of sludge application as a means of farm land fertilization appears to cause no increase in the number of parasitic ova and larvae when compared to control pasture areas. Also, the number of free-living ova and larvae greatly outnumbered the parasitic forms observed on all farms. This is consistent with expectations because care taken in avoiding manure piles during collection decreased the chance of recovery of cattle intestinal parasite eggs normally passed in the feces. Although this study does not eliminate the possibility that parasitic infections (e.g. cysticercosis) might occur in cattle following grazing on sludge treated pastures, the current practice of preventing cattle from grazing sludge treated pastures for four weeks after sludge application is a safeguard against unusual parasite exposures that might occur.

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TABLE 14-1. RESULTS OF PARASITOLOGIC EXAMINATION OF FORAGE SAMPLES COLLECTED FROM SLUDGE TREATED AND CONTROL PASTURES, FARM NO. 4018.

Time (days)	Treatment	Rainfall (inches/wk)	No. of larvae counted	No. per kg. of dry forage
0 (pre-sludge)	Sludge	nr*	18	130
0 (pre-sludge)	Control	nr*	12	80
7	Sludge	1.40	55	450
7	Control	1.40	56	400
14	Sludge	1.40	6	44
14	Control	1.40	10	80
28	Sludge	1.05	107	1,180
28	Control	1.05	129	710

* nr - not recorded; no data collected.

TABLE 14-2. RESULTS OF PARASITOLOGIC EXAMINATION OF FORAGE SAMPLES COLLECTED FROM SLUDGE TREATED AND CONTROL PASTURES, FARM NO. 4017.

Time (days)	Treatment	Rainfall (inches/wk)	No. of larvae counted	No. per kg. of dry forage
0 (pre-sludge)	Sludge	nr*	8	60
0 (pre-sludge)	Control	nr*	5	30
7	Sludge	nr*	nr*	nr*
7	Control	nr*	nr*	nr*
14	Sludge	1.60	89	700
14	Control	1.60	129	1,300
28	Sludge	1.20	19	120
28	Control	1.20	38	210

* nr - not recorded; no data collected.

TABLE 14-3. RESULTS OF PARASITOLOGIC EXAMINATION OF FORAGE SAMPLES COLLECTED FROM SLUDGE TREATED AND CONTROL PASTURES, FARM NO. 3005.

Time (days)	Treatment	Rainfall (inches/wk)	No. of larvae counted	No. per kg. of dry forage
0 (pre-sludge)	Sludge	nr*	11	80
0 (pre-sludge)	Control	nr*	7	60
7	Sludge	1.10	11	110
7	Control	1.10	21	200
14	Sludge	1.80	13	130
14	Control	1.80	19	300
28	Sludge	1.00	41	270
28	Control	1.00	19	150

* nr - not recorded; no data collected.

TABLE 14-4. NUMBER OF PARASITIC AND FREE-LIVING LARVAE EXAMINED IN ALIQUOTS OF FORAGE SAMPLES COLLECTED FROM SLUDGE TREATED AND CONTROL PASTURES DURING ALL SAMPLING PERIODS.

Farm No.	No. of Larvae	
	Parasitic	Free-living
4018	5	32
4017	5	17
3005	5	21

SLUDGE DISPOSAL ON FARM LAND: AN EPIDEMIOLOGIC EVALUATION OF THE RISK OF INFECTION

Vincent V. Hamparian[1], John H. Hughes[1], Abramo C. Ottolenghi[1]
Frank A. Kapral[1], Melvin L. Moeschberger[2], and Richard Lanese[2]

Department of Medical Microbiology and Immunology[1]
Preventive Medicine[2]
The Ohio State University
Columbus, Ohio 43210

ABSTRACT

During the period of this study, 307 sludge samples collected from 4 sewage treatment plants in 3 different geographic areas were tested for the presence of viruses. One or more viruses were isolated from 211 (69%) of the 307 samples. Of these, 130 yielded one virus, 2 viruses were isolated from 76 samples and 5 samples yielded 3 viruses. Excluding the polioviruses, 22 different enterovirus serotypes were isolated. Salmonellae were isolated from 50-311 sludges (16%) with rates varying by year and site.

Of 297 viruses isolated from sludge, 179 (60%) were recovered in RD cells, 45 (15%) in BGM and 77 (26%) in HeLa M cell cultures. These results emphasize the importance of using more than one type of cell culture when attempting to isolate viruses. Twenty-one different serotypes of Salmonella were isolated from sludge. Salmonella infantis was isolated most frequently.

Eighty two sludge samples were examined for parasite ova. Toxocara ova were the only animal parasite ova seen. They were detected in 5 samples.

Using sera from serial blood samples and 23 enteroviruses in neutralization tests, 124 rises (4-fold or greater) were detected in 67 people. Of these, 69 rises (infections) occurred in 34 subjects residing on sludge-receiving farms and 55 occurred in control subjects. The rises were rather evenly distributed between the coxsackieviruses (51%) and the echoviruses (48%). Few rises (8) in antibodies to Salmonellae were seen in the subject population during the course of the study. Here also the rises were evenly distributed between control and sludge exposed individuals.

To determine whether exposure to sludge under the conditions of this study caused a higher frequency of enteroviral illness, matched pair logistic regression analysis was done of 4-fold increases in antibody titer to any of 23 viral antigens. These analyses were done on paired farms after the first and second applications of sludge and after the entire 3 year period of the study. The results indicate that exposure to sludge is not related to differential increases in antibody titer. The findings are consistent and in favor of the null hypothesis. Furthermore, McNemar's Test was used to look at the health effects of sludge exposure, taking into account the total experience of each cohort between the initial sludge application and the end of the project. The results of these analyses also are consistent with those obtained by multiple logistic regression analysis.

INTRODUCTION

Under the auspices of the U.S. Environmental Protection Agency, a five year study involving the controlled application of sewage sludge to Ohio farmlands has been completed (Sludge Demonstration Project, Ohio Farm Bureau Federation, Inc.). Since no comprehensive, prospective, epidemiologic investigations have been done to directly evaluate the human health risks from sludge used in this manner, the Ohio Farm Bureau's Demonstration Project has provided a unique opportunity to carry out this kind of investigation. Control farms and farms receiving sludge located in Medina, Franklin, Pickaway and Clark counties were included in the study. Some subjects were followed less than the planned three years because they were recruited into the study to replace individuals that had dropped out. In addition, Clark County was incorporated late into the study to replace Pickaway County when that County's Health Department stopped the disposal of sludge on farmlands. The human health portion of this project was approved by the appropriate Institutional Human Subject Review Committee of The Ohio State University and informed consent was obtained from all subjects prior to their inclusion in the study.

Since it is well established that sewage sludge can contain a variety of human pathogens including various viruses, salmonella and parasite ova (Kowal, 1982), the aims of this part of the study have been to monitor individuals on both control and sludge-receiving farms for the occurrence of viral, bacterial, and parasitic enteric infections. The study was designed in a manner that would provide primary emphasis on the detection by serologic means of enterovirus infections in farm families. The rationale for this emphasis is based upon the fact that humans can be infected when exposed to small amounts of enterovirus. It has been reported that as little as one infectious unit of poliovirus in cell culture medium is sufficient to infect a human (Katz and Plotkin, 1967). In addition, two plaque forming units (pfu) of poliovirus vaccine virus were capable of infecting 67% of vaccinated infants, while 20 pfu of virus infected 100% of vaccinees (Koprowski, 1956). Furthermore, it has been demonstrated that humans could be infected with 18 TCD_{50} of coxsackievirus A21 when administered in an aerosol (Gerone _et al._, 1966). Westwood and Satter (1976) have summarized most of the evidence indicating that one virion is capable of establishing an infection in a mammalian host. There is therefore reasonable evidence indicating that a very small dose of an enterovirus can cause infections in humans.

The advantages and limitations of using serologic methods as an epidemiologic tool to detect infections have been reviewed elsewhere (Miday, 1980). As opposed to questionnaires, serology supplies an objective measure of infection including subclinical infections. It also provides information on the past experience of individuals with microbial antigens and on their susceptibility to infection. The most important limitations of serology include the need to test for infections with the correct agent at the correct time and to test for infections by enough different agents to be able to assess risk. These problems were obviated in this study by 1) testing for infection with 23 different enteroviruses and hepatitis A virus; 2) by obtaining serum samples prior to sludge application and then three times per year and 3) monitoring the sludge applied to land for the presence of enteroviruses to assure that we were testing for infections using viruses commonly present in sludge applied to land.

Excluding the polioviruses, there are approximately 60 enteroviruses that can be propagated in cell culture systems and are therefore available to monitor human infections using serum neutralization tests performed in cell cultures. Thus, about 35% of the recognized enterovirus serotypes were used in our surveillance system for the detection of enteroviruses. Enterovirus infections were detected by searching for significant serum neutralizing antibody increases to 23 enteroviruses among serial serum samples taken before and after the application of sludge to farmlands.

Control and sludge subjects were bled every four months for as long as they were in the study. In addition, study subjects were monitored for hepatitis A virus infections by testing the serial serum samples for the development of specific antibody (conversion from negative antibody status to positive). Since most enterovirus and hepatitis A virus infections do not result in frank disease, serology is a sensitive and relatively practical method of detecting these infections. To assure that at least a proportion of the viruses used in our neutralization tests were present in sludge applied to farmlands, we also monitored sludge from all study sites on a biweekly basis for the presence of enteroviruses. When necessary and if appropriate, viruses frequently found to be present in sludge samples were added to our neutralization test system, since such viruses could be excellent indicators of the spread of viruses from sludge to humans. Echovirus 7 is an example of a virus we added to our test system because of its presence in many samples of sludge. All sera were tested against the newly added enteroviruses. Viral isolation attempts also were done on three stool specimens collected annually from each subject with one sample always collected during the summer.

To detect enteric bacterial infections, the stool specimens obtained three times a year from all subjects were tested for enteric bacterial pathogens. In addition, the most recent sera from all subjects were tested for the presence of agglutinins to salmonella types B,C,D and E. If positive, the original base line sera were tested to determine if a serologic conversion had occurred or if the agglutinins were present at the beginning of the study. The sludge samples described above also were tested for enteric bacterial pathogens by techniques capable of detecting a minimum of 11 salmonellae per ml of sludge. This information allowed us to compare the organisms present in sludge with those isolated from the stools of subjects. In addition, stool and sludge samples were tested for the presence of parasite ova. All tests involving the health portion of this study have been completed and the data collected comprise the subject of this part of the report.

COLLECTION OF SPECIMENS

Sludges. Sludge samples were collected biweekly from the M300 and M500 plants in Medina County, Ohio, the Jackson Pike Plant of Columbus, Ohio and the sewage treatment plant of the city of Springfield, Ohio. Samples of 1.5 to 2.0 liters were collected in sterile plastic bottles at the plant site from which trucks were loaded. The bottles were tightly sealed, labelled and stored at 4°C. The next morning, the samples from Medina and Springfield were placed in insulated boxes on wet ice and shipped to the laboratory via a commercial carrier. The Columbus samples were picked up by laboratory personnel on the same day of collection. Attempts to isolate bacteria and processing of sludge for the isolation of viruses were done on the day of arrival of the samples in the laboratory. Eluates from sludge samples for virus isolations were stored at -20 C until used.

Stool Samples. These were obtained three times per year from both controls and sludge subjects. The samples were obtained approximately at four month intervals with at least one sample being obtained during the Summer or early Fall. The samples were collected in plastic containers which were sealed and either placed in a cooler with wet ice and delivered to the laboratory the same day or refrigerated overnight and delivered the next day. The samples were tested for bacterial pathogens and processed for viral isolation on the day of arrival. The processed stools were stored at -20°C until used for isolation. A portion of each unprocessed stool sample was placed in neutral formalin and stored at 4°C until examined for the presence of parasite ova.

Human Sera. A baseline serum sample prior to land application of sludge was obtained from each subject. Subsequently, three serum samples per year were obtained from each individual throughout the course of the study. When blood samples were obtained from subjects outside of the Columbus area, they were centrifuged on the same day by a local commercial laboratory, the sera were transferred aseptically to sterile vials and transported on wet ice.

A. Bacteriological Studies

MATERIALS AND METHODS

Isolation of Salmonellae and Shigellae from Sludge (Fig. 1). Unless otherwise noted, all media were obtained as dehydrated bases from Difco, Detroit, Michigan.

Day 1

Sludge was inoculated directly onto plates of Hektoen Enteric agar (HEK), MacConkey Agar (MAC) and Xylose-lysine deoxycholate agar (XLD) by means of swabs. The plates were then streaked for isolation. Impregnated swabs were also added to tubes containing 10 ml of Selenite or GN (BBL, Cockeysville, Maryland) broths. In early samplings, Tetrathionate broth (BBL Cockeysville, Maryland) tubes were also inoculated. This procedure was later abandoned because recovery was no better than with the other two enrichment media. Plates and enrichment broths were incubated at 37°C, the plates for 24 hrs; the broths for 24 and 48 hrs.

Day 2

H_2S producing colonies were picked from HEK and XLD agars and tested on biochemical media. Lactose negative colonies were tested for oxidase activity and the negative ones were tested on biochemical media. The enrichment media cultures were streaked on HEK, XLD and MAC agars and incubated for 18-24 hours at 37°C.

The biochemical media used for screening were Triple Sugar Iron agar (TSI), Lysine-indole agar (LIA) and urea agar. Colonies were also suspended in 0.3 ml of saline to which a disk of ortho-nitrophenyl galactoside (ONPG) was added. All tubes were incubated for 18-24 hrs at 37°C.

Day 3

All plates streaked from the enrichment broths were examined as indicated above and suspected colonies were inoculated into biochemical test media as described above. All tubes of biochemical reactions were examined and where the reactions indicated the possible presence of salmonellae, API strips were inoculated according to manufacturer's directions. The enrichment cultures were again streaked on HEK, XLD and MAC plates.

Day 4

Plates streaked for isolation on day 3 were examined as described above. Biochemical tests and API inoculations were performed on all suspected colonies as indicated above. Any sample which was identified as a Salmonella was transferred to 3 TSA slants to be used for serological identification and for submission for confirmation. All salmonellae isolated were tested for group antigens using group specific sera (Difco) in agglutination reactions and then submitted to the Ohio Health Department Laboratory for speciation.

Antibiotic sensitivity testing and biochemical confirmation were performed in the Clinical Bacteriology Laboratories of The Ohio State University Hospitals. All confirmed salmonellae were repurified and freeze dried in skimmed milk for deposit in the collection.

Isolation of Salmonellae and Shigellae from Stool Specimens. A 10% suspension of the stool specimen (under 24 hrs. old) was cultured and handled as described above for sludge. A total of 1,821 stool samples were tested.

Recovery of Salmonellae and Shigellae from Seeded Sludge Samples. Three different strains of Salmonella were used. For the early experiments, a stock of S. typhimurium from our laboratory collection was used. For later experiments, a strain of S. typhimurium and a strain of S. paul both isolated from sludge were used. The shigella strain used was one from our departmental collection. Two methods were used to test the effectiveness of our recovery system.

Seeding of Samples at Plant. Two 1,200 ml samples of sludge from the Medina 300 plant were seeded with 2×10^7 shigella and 1×10^7 salmonella to give a final concentration of 1.6×10^4/ml shigella and 8×10^3/ml salmonellae.

Seeding of Samples in the Laboratory. The salmonella strain to be used was inoculated into 3, 9 ml samples of freshly received sludge to give final concentrations of ca. 2×10^3, 2×10^2 and 20 CFU/ml and then processed together with the routine samples. Experiments were conducted with sludge from each site in the health part of the study.

Isolation of Campylobacter sp. from Sludge. For all recovery experiments and for control purposes, we used a patient isolate of Campylobacter foetus ssp. jejuni obtained from the bacteriology laboratories of The Ohio State University Hospital. Sludge was swab-inoculated on triplicate Campy Blood Agar Medium (CBP) plates (Remel Corporation) and streaked for isolation. Plates were incubated at 25°C, 37°C, and 42°C in stainless steel jars in an atmosphere consisting of 5% O_2, 10% CO_2 and 85% nitrogen. Control plates of C. fetus ssp. jejuni were included in each jar. The cultures were examined at 24 hrs and 48 hrs and colonies were picked and tested for catalase and oxidase activity. Positive colonies were further tested for motility, H_2S production, and resistance to nalidixic acid and cephalothin.

Recovery of Campylobacter from Seeded Sludge. Campylobacter fetus s.s. jejuni was grown for 48 hrs on CBP. The organisms were suspended in 3 ml of freshly boiled thioglycollate broth. Ten fold dilutions were made and calibrated loop counts were performed on the suspension by inoculation in triplicate with a 0.001 ml calibrated loop. Preliminary counts indicated that 1 ml of the undiluted suspension contained ca. 2×10^7 CFU/ml so appropriate volumes were inoculated in duplicate into 9 or 9.9 ml of sludge suspension to obtain suspensions ranging from ca. 2×10^6 to 2×10^2 CFU/ml. Mixing of the suspensions was accomplished by bubbling the mixture with the gas used for incubation described above. The seeded sludge suspensions were tested as described above for the isolation of campylobacter. To test for survival of the organisms in sludge, the suspensions were stored at 4°C for 1 hr, 24 hrs, 4 days and 7 days prior to plating and incubated as described above. Because of the relative thickness of the sludge obtained from the Columbus Jackson Pike Plant, this sludge was diluted 1:4 with sterile isotonic saline before addition of the bacteria.

Isolation of Salmonellae from Stools. Stool suspensions (10%) were processed according to the protocol described for sludge specimens. When a patient specimen was positive, repeat cultures were taken every 3 weeks until two negative cultures were obtained.

Detection of Antibodies to Salmonellae Antigens.. The last available serum from each individual was tested for antibodies to Salmonella groups B, C, D, and E by the rapid slide test using Bacto-Widal Salmonella O antigens (Difco, Detroit, Michigan). 0.04 ml of serum was used for the determination (equivalent to a 1:20 correlated dilution). Results were scored from negative to 4+ depending on the degree of clumping that was observed. If the most recently obtained serum of an individual was positive at a level of 1+ or more, the baseline and subsequent sera were tested until a positive serum was found. If the baseline serum of an individual was also positive for the given group(s) antigen, no additional sera were tested.

RESULTS

Recovery of Shigellae, Salmonellae and Campylobacter from Seeded Sludge. Both samples of sludge seeded at the Medina 300 plant yielded salmonella. Tables 15-1 - 15-4 show the results of the experiments designed to test the effectiveness of recovery of organisms from sludge seeded in the laboratory. Results were similar for both Medina plants. Salmonellae were detected following incubation in enrichment broth for up to 7 days even when as few as 11 CFU/ml were present, while direct plating resulted in recovery on HEK agar only at 10 times higher levels and then only when the organisms had been in the sludge for only 24 hrs. In addition, in one instance when using Springfield sludge which contained group C salmonellae for seeding experiments, both the seeded group B Salmonella and the group C Salmonella were recovered. Table 15-5 shows the frequency of isolation of salmonellae following direct plating or enrichment of the sludge. Twenty-two recoveries from sludge samples and from 3 stools were analyzed to determine on which media the salmonellae colonies were detected. In only 5 cases were the organisms recognized on primary isolation plates. Two were human isolates and the others were one each from the two Medina plants and the Columbus plant.

No shigellae were recovered in our seeding experiments even when organisms were added to a final concentration of 1.6 x 10^4 CFU/ml. Campylobacter was recovered when ca. 150 CFU/ml were present (Tables 15-6 - 15-9). Recovery was successful even when the organisms had been held in sludge for 7 days at 4°C.

Attempts to Isolate Shigellae from Sludge and Stool Samples. No Shigellae sp. were isolated from any of the sludge or stool specimens tested.

Isolation of Campylobacter sp. from Sludge. No Campylobacter sp. were isolated from 99 samples of sludge tested during the period September 1980 - June 1981. Forty-one of the samples were from the Columbus plant, 18 from each of the Medina plants and 22 from the Springfield plant.

Isolation of Salmonellae from Sludge. A total of 50 isolations of Salmonellae sp. were made from the 311 samples of sludge (16%) tested from all sources during the period of study. The frequency of isolation varied by year and by site. See Tables 15-10 and 15-11. Isolation was most frequent from sludge samples obtained from the Columbus treatment plant (25%) and least frequent from the Springfield plant (7%). See Table 15-12.

Twenty-one different serotypes and 5 nontypable strains were isolated (Table 15-13). The most frequently encountered organism was Salmonella infantis isolated on 8 occasions; Salmonella St. paul, S. typhimurium and S. agona were the next most frequently isolated serotypes (4 times each).

The distribution of isolations from a given plant did not follow any particular pattern except that there appeared to be an increased isolation rate during the first quarter of each year (Table 15-14). An overall Chi-square test of the data showed a borderline P value ($0.1 > P > 0.05$) so the data was subjected to an analysis of residuals (Everit, 1977) which indicated that a significant deviation existed in the number of isolations made during the first quarter (2.43 S.D.). A 2 x 2 Chi-square analysis of the isolations during the 1st quarter vs. all other quarters combined yielded a $P = <0.02$ indicating that substantially more isolations of salmonellae were made during the first quarter than would have been expected.

From inspection of the data in Table 15-14, it appeared that the increase was primarily due to the isolations from the Columbus plant. To test this hypothesis, the 1st quarter data from Columbus was tested in a 2 x 4 Chi-square test against 1st quarter data from the other sites. The overall test gave a $P = <0.05$. When Columbus' first quarter data was compared in a 2 x 2 Chi square test with the combined data from the other 3 quarters in Columbus (Table 15-15), a significant difference was also noted ($P = <0.05$).

Antibiotic Sensitivity Patterns of the Salmonellae Isolated from Sludge. Table 15-16 presents the distribution of the minimum inhibitory concentration (MIC) of given antibiotics for most of the strains isolated from sludge. A few strains were lost before the antibiotic sensitivity could be tested. In general, most strains showed low MICs for most antibiotics. Table 15-17 lists the 5 strains which showed high MICs to one or more antibiotics.

Isolation of Salmonellae from Individuals. Table 15-18 indicates the isolations which were made from individuals. One individual was positive following repeated cultures.

Antibodies to Salmonellae in the Farm Population. The serologic status of the subject population at the end of the study is shown in Table 15-19. Individuals with no antibodies at the end of the study were for our purposes assumed to have been negative at the start of the study.

A statistical analysis by the Chi-square test of the data in Table 15-19 indicates a significant deviation of the data for the incidence of antibodies against Group C salmonellae from a normal distribution ($P = <0.001$).

DISCUSSION

Enteric Bacteria in Sludge. The procedure used by us for the isolation of salmonellae from sludge is similar to that described by Dudley et al., 1980, in that we too used selenite broth as one of our enrichment media and incubated the enrichment at 37°C rather than at 42°C as suggested by Spino, 1966; Harvey and Price, 1968; Cheng et al., 1971, and Yoshpe-Purer et al., 1971. Edgar and Soar 1979, reported in a study comparing methods for the isolation of salmonellae that several methods described in the literature produced different results not only because some of the media used were inhibitory for the organisms, but also because in some instances, certain of the media coupled with higher temperatures of incubation were inhibitory. These authors reported that Brilliant Green Agar was the best plating medium in their hands.

Although we did not use brilliant green as the plating medium, we obtained good growth and differentiation with HEK and XLD plating media, the former giving us the better results. The sensitivity of our isolation system was sufficient for our purposes in that we were able to detect 11 CFU seeded in a 1 ml sample of sludge (1.9 - 6.0% solids). Dudley et al., 1980, reported a detection limit of >2<24 CFU/g total solids. In studies directed to the determination of the Most Probable Number (MPN) of salmonellae in sludges from different sources, Jones et al., 1980, were able to find organisms in sludges containing as little as 0.3 salmonellae CFU/100 ml. The methodology used by Jones and his colleagues was much more extensive than ours in that multiple enrichment systems were used. This question will be discussed below in conjunction with a discussion of minimal infective doses.

As reported by Dudley et al., 1980, we also were unable to detect Shigella sp. in the sludge samples. Our recovery experiments suggest that shigellae do not survive well in sludge, which would decrease the probability of this organism being present in the sludge applied to land. However, we can not exclude the possibility that the methodology used by us and other workers is not sufficiently sensitive to permit the isolation of shigellae.

Because of this lack of isolation of Shigellae sp., organisms from any of the sludges and the relatively high numbers required for recovery in our seeded experiments, we are unable to reach any conclusion as to the presence or absence of this pathogen or as to the relative risks involved.

Our inability to recover Campylobacter sp. from samples of sludge despite recovery of the organism from our seeded experiments even after 7 days of contact at 4°C indicates that the organism if present in sludge, is in such low concentrations as to be undetectable by our methodology. The high sensitivity that this organism displays to oxygen might make its presence unlikely in aerobically digested sludges.

The different rates of isolation of salmonellae from the different sites as shown in Table 15-12 is not surprising. Similar results have been obtained by Danielsson, 1977, Dudley et al., 1980, and Jones et al.,1980. The rate of isolation depends on the initial number of salmonellae present in the raw sewage and the effects of the particular treatment. Our lowest frequency of isolation, 7% from the sludge from Springfield was similar to the rate of 8% reported by Danielsson (1977) in her studies in Sweden.

We are unable to relate the higher number of isolations from the Columbus plant during the first quarter of each year to any specific condition at the plant. Carrington et al., 1982, showed that the inactivation of salmonellae in anaerobic sludge was affected by temperature during digestion so we subjected the frequency of isolation data to statistical analysis reasoning that in the first quarter of the year, temperatures would tend to be lower and thus the organisms might be protected. Examination of the data however, indicated that organisms were isolated in no discernible pattern. The temperature of the digestors varied between 30-35°C during the course of the study.

Based on our recovery experiments from seeded sludge samples, the relatively low rate of salmonellae isolation and the fact that most of our samples required enrichment for isolation, it is likely that the number of organisms in the sludges used in this study was usually less than 11-40 CFU/ml. When present in higher concentration, there probably were not more than 280 CFU/ml since in only a few cases were we able to recover organisms directly from plated material without previous enrichment. This concentration of organisms coupled with the low rate of sludge application to land (4-10 metric tons of solids/hect) would make it most unlikely that an individual would assimilate an infective dose.

There is also the concern that the lack of isolation of salmonellae from sludge might not be a sufficient criterion for the assessment of risk since there is always the potential for repopulation of the sludge on standing before it is delivered to the fields. In this context, Russ and Yanko, 1981, reported that in composted sludges (not exactly our case), the pathogen repopulated readily if the moisture content was at least 20%, if the temperature was in the mesophilic range and if the carbon-nitrogen ratio was in excess of 15:1. The two first conditions exist in this present study but the carbon/nitrogen ratio is well below this as has been seen in an earlier section of this report.

The concept of infective dose under natural conditions is difficult to establish, although Cliver, 1980, reported that salmonella gastroenteritis occurred in adults and children eating chocolate contaminated with <1 to 100 *S. eastbourne* organisms per 100 g. In another case, Lang *et al*, 1967, reported that 15,000 cells of *S. cubana* were required for illness in patients ingesting contaminated carmine dye. Another study of a naturally occurring infection, that of infection with *S. typhimurium* contaminated drinking water in Riverside, California in 1965 (Boring *et al*., 1971) indicated that the water contained 17 CFU/l. The authors however, advise caution in interpreting these data for the determination of infective dose because of some of the procedures used in sampling the water.

Ayanwale *et al*., 1980, report feeding goats for 17 months on corn silage grown on land fertilized with sludge from which *S. newport* C_2 had been isolated. No rises in antibodies to the organism were seen. Under controlled experimental conditions, infective doses for *Salmonella sp*. other than *S. typhosa* have been reported to vary between $10^5 - 10^8$, although in some experiments, 10^6 did not cause either disease or infection (summarized by Kowal, 1982). Because of these considerations, we believe that the effort and expense involved in determining the MPN of salmonellae in a given sludge sample routinely is not justified since our results indicated that large amounts of the sludge would have to be consumed before infection with this organism could be expected to occur. More refined and definitive quantitative results would not significantly alter the assessment of risk.

Serologic Examination for Antibodies to Salmonellae. Because agglutinating reactions to salmonellae antigens may be a reflection of cross-reactivity with antigens from other *Enterobacteriaceae*, we did not conduct as rigorous a survey of agglutinating antibodies as we did for the neutralizing antibodies against the enteroviruses. Our methodology was not designed to detect rises and falls in the antibodies to the different groups of salmonellae but rather on an all or none basis with a titer of 1:20 as our baseline. Using these criteria, it can be seen that there is a significant difference in the antibody patterns detected in different locations and in different families. We did not have enough data for a valid statistical analysis of relationship between the occurrence of antibodies to salmonellae and the presence of a significant number of animals on a farm. Similarly, because of the relatively low number of individuals with antibodies, it is not possible to determine whether there was a significant difference at the end of the trial between the individuals on farms where sludge was placed and individuals on control farms.

On an episodic basis, there appear to be some families with a higher prevalence of antibodies to salmonellae. See Table 15-21. The small number of apparent conversions also precludes a valid statistical analysis. What is of interest however, is that when the most recent positive sera from given individuals were matched with baseline sera (usually collected 3 years earlier), the same pattern of reactivity was observed in most cases. The low number of actual conversions and some apparent anamnestic responses do indeed strengthen the possibility that there is a low incidence of human salmonellosis in the population studied. In conclusion, we did not detect a sufficient number of infections with salmonellae during the study to be able to analyze the data statistically.

CONCLUSIONS

1. Salmonallae do not pose a significant risk to the health of farm families exposed to sewage sludge applied to farm land under the conditions used during this trial.

2. The number of salmonellae present in the sludge samples used does not appear to be sufficient to cause infection as measured by the rapid agglutination test.

3. *Shigellae sp*. and *Campylobacter sp*. were not found in sludge under our conditions.

B. Parasitology

Examination of Stools for Parasites and Ova. The procedure used for examination of stools for the presence of ova and parasites was the direct examination method (Melvin and Brooke, 1974). Fecal material was added to 20 ml of distilled water in a 40 ml conical centrifuge tube and vortexed and shaken until a smooth suspension was formed. The mixture was centrifuged at 8900 x g for 2 minutes at 4°C in a swinging bucket rotor. Enough stool was used to provide about 15 ml of sedimented stool material. The supernate was discarded and for direct examination, a drop of the washed fecal material was mixed with a drop of distilled water on a microscope slide and examined under a microscope.

To determine the sensitivity of our methods, aliquots of a known negative stool sample were seeded with various amounts of ascaris ova from a suspension containing a known number of ova. Individual samples were seeded with amounts calculated to give final numbers of 700, 350, 175, 88, and 44 ova/ml. A negative control also was included. The stools were then processed with and without concentration procedures which included the zinc sulfate and the formalin-ether concentration procedure (Melvin and Brook, 1974) for comparative purposes. By the direct method, with 88 ova/ml in feces, ascaris ova were detectable in 50% of the preparations and with 175 ova/ml in all of the preparations. Similar results were obtained with the formalin-ether concentration technique. No ascaris ova could be detected at the highest concentration used (700 ova/ml) with the zinc sulfate concentration procedure. Even though the zinc sulfate procedure was not an efficient means of detecting ascaris ova, it is useful for other ova and this method and the direct method were used to test 407 stool samples obtained from the different test sites for ova and parasites.

Examination of Sludge for Ova and Parasites. The method used for these tests essentially is that described by Meyer et al., 1978. One hundred ml of 2.6% hypochlorite solution were added to 75 g (wet weight) of sludge in a 250 ml centrifuge bottle and mixed vigorously. After the foam had subsided, additional hypochlorite solution was added to give a final volume of 225 ml and the mixture was kept at room temperature for 1 hour. Subsequently, the suspension was centrifuged at 800 x g for 2 minutes at 4°C, the supernate was decanted and 2 ml of an anionic detergent (7X, Linbro Sci. Inc., Hamden, Conn.) was mixed into the pellet. Distilled water was added to a final volume of 225 ml and the mixture was centrifuged as described above. The pellet was resuspended in distilled water again and recentrifuged. This procedure was repeated and the resulting sediment was resuspended in zinc sulfate solution (specific gravity 1.225) and centrifuged at 800 x g for 2 minutes at 4°C. After standing for a few minutes, the surface of the supernate was aspirated and examined as described above for feces.

Tests for sensitivity for the detection of ova were repeated as described above for stool samples. To detect ova on a regular basis in a 30 g (wet weight) sample of Medina Sludge, 3000 ova were required.

RESULTS

Parasitology - Stools. See Table 15-22 for the stool samples tested according to year collected, geographic source and study status. Twenty-six percent of the 1,556 stools collected were tested and found to be negative. Most of the stools tested were from the last 2 years of the study on the rationale that if ova from sludge were capable of infecting humans, these stools would be most likely to contain ova and parasites.

Parasitology - Sludge. Eighty-two samples from 4 plants were examined. The majority of ova present were mite ova of the genus Orbatiol. In addition to the ova, some hatching and apparently free-living mites also were seen. Some sludge samples from Columbus contained Toxocara ova. No ova or parasites were detected in the small number of sludge samples examined from the Springfield Plant. See Table 15-23 for these results.

DISCUSSION

Under the conditions of this study, the monitoring of stool samples for ova and parasites was of no value for evaluating the health effects of sewage sludge applied to farmlands. Over 400 stool samples from sludge exposed (234) and control subjects (173) collected primarily during the last two years of the study, were negative for parasites and ova (Table 15-22). This is not a surprising finding because the frequency of *Ascaris lumbricoides* infections of humans in this country appears to be very low. In 1976, about 2% of over 400,000 stools submitted to state health laboratories were positive for *A. lumbricoides* ova (Cross, 1982). It is therefore likely that the prevalence of such infections in a normal population of farmers in Ohio would be even lower unless of course, exposure to sewage sludge containing enteric parasites was an efficient means of spread to humans. Although it has been reported that ova from enteric parasites are present in sludge in this country and can remain viable during digestion (Black et al., 1982), the only ova we were able to identify in sludge were *Toxocara* ova which were detected in 5 of 27 sludge samples from the Columbus plant (Table 15-23).

It is possible that the methods we used were not adequate to detect ascaris ova in sludge. To detect such ova on a regular basis in seeded sludge, required the presence of 3000 ova in 30 g (wet weight) of sludge. In stool samples however, we could detect ova when present at a concentration of only 88 ova/ml.

C. Virus Studies

Materials and Methods

Cell Cultures. Primary cynomolgous and green monkey kidney tube cultures and cell suspensions were obtained from a commercial source. HeLa M cells (Hamparian, 1979) were from the cell bank of this laboratory. The BGM line of African green monkey cells (Barron *et al*., 1970) originally was obtained from Dr. A.L. Barron. The RD line of human rhabdomyosarcoma cells (McAllister *et al*., 1969) was supplied by Dr. Nathalie J. Schmidt. All cell cultures were propagated according to standard procedures (Schmidt, 1979). The residual trypsin (0.25%) method was used to disperse cells for serial cultivation. Growth medium for all cultures consisted of Eagle minimum essential medium (EMEM) in Earle balanced salt solution (EBSS) containing a final concentration of 10% fetal bovine serum, 50 mcg of gentamycin per ml and 12 ml of 7.5% $NaHCO_3$ per liter. Prior to inoculation and with the exception of the RD cells, all cultures were placed on maintenance medium consisting of the above except that the fetal bovine serum was reduced to 2% and the $NaHCO_3$ was increased to 30 ml per liter. For reasonable maintenance (5-7 days) of RD cells on Eagle MEM in Earle salt solution, the amount of 7.5% $NaHCO_3$ in the maintenance medium had to be kept at 12 ml per liter.

Cell lines were preserved by suspension in Eagle MEM with a final concentration of 10% fetal bovine serum and 10% dimethylsulfoxide and storage in liquid nitrogen. The details of the methods for preservation and storage of cells have been described (Schmidt, 1979).

Viral Isolations from Sludge. The method used to isolate enteroviruses from sewage sludge included an elution procedure and a disinfection step. No concentration procedure was used because a) the amount of eluent used for each 6 g of sludge solids was only 5 ml and b) 30 ml of each eluate (at least 30% of the sample) was inoculated into cell cultures.

A technique utilizing beef extract was employed to elute viruses from sludge particulates. Beef extract is a commonly used and relatively efficient eluent for this purpose (Brashear and Ward, 1982; Farrah et al., 1981; Glass, et al., 1978; Landry et al., 1978; Nielsen and Lydholm, 1980; Sattar and Westwood, 1976; Wellings et al., 1976). Sludge samples that were mostly liquid (3-5% solids) were centrifuged for 30 minutes at 1800 x g at room temperature. The supernatant fluids were discarded. The volume of sludge centrifuged usually yielded about 100 g (wet weight) of sludge solids. Sludge samples from the Columbus plant which are dewatered by centrifugation, contain about 18% solids and had the consistency of soft putty. For such samples, 100 g of the sludge were weighed out. Five ml (sterile) of 3% beef extract in distilled water and containing 0.1% sodium dodecyl sulfate was added for each 6 g of solids. The pH of the eluent was 7.5. The mixture was stirred with a magnetic stirrer for one hour at room temperature to elute viruses. The mixture was then clarified by centrifugation at 4°C for 30 minutes at 800 x g. The supernatant fluids were drawn off and disinfected by adding 1 ml of chloroform for each 20 ml of eluate and incubating the mixture at room temperature for 30 minutes with intermittent shaking. The chloroform-eluate mixture was separated by centrifugation at room temperature for 30 minutes at 800 x g. The supernatant fluid was stored at -20°C until inoculated into cell cultures. Figure 2 summarizes the isolation procedure.

Initially RD, HeLa, BGM and primary cynomolgous monkey kidney cultures (CMK) were used; however, after viral isolation attempts were done on 34 sludge samples from the Medina 300 plant, 33 from Medina 500, 18 from Columbus and 5 from the Springfield sewage treatment plant, it became clear that the primary monkey kidney cultures were not of value for isolating enteroviruses. The occasional isolates obtained in CMK cultures were either reoviruses or enteroviruses also isolated in RD or BGM cell cultures. Thus, because of the high cost and poor viral yield of primary CMK cultures, they were dropped in late 1979-early 1980 from the spectrum of cells used for isolation of viruses from sludge. Two 16 oz. flasks of each cell culture were inoculated with 5 ml each of a sludge eluate. Because of the toxicity of most sludge eluates, all cell cultures were inoculated in the presence of growth medium. Attempts to inoculate cultures containing less than 10% fetal bovine serum in the medium or with no medium, usually resulted in rapid destruction of cell sheets. The inoculated cultures were incubated for one hour at 36°C to allow for viral adsorption, the supernatant fluids were discarded and the cultures were refed with 35 ml of maintenance medium. This procedure virtually eliminated problems with the toxicity of sludge eluates. Uninoculated control cultures were included with each isolation attempt. The cultures were observed for cytopathic effects (CPE) for ten days and all positive cultures (75% or more involvement of the cell sheet) were stored at -20°C until identified.

Viral Isolations from Stool Samples. A 10% suspension (wt/vol) of stool sample was prepared in PBS and centrifuged at 1000 x g for 20 minutes. Three ml of the supernatant was mixed with 1.0 ml of an antibiotic solution containing 3,000 units of penicillin and streptomycin, 1,600 mcg of chlortetracycline and 353 mcg of amphotericin B. The mixture was incubated at room temperature for one hour with occasional shaking and centrifuged at 4°C for one hour at 1000 x g. The supernatant fluid was stored at -20°C until inoculated into cell cultures. Primary green monkey kidney, RD and BGM tube cultures were placed on maintenance medium and two tubes of each kind of cell culture were inoculated with 0.2 ml of a stool sample. Cultures were incubated at 36°C on a roller drum and were observed for CPE for 10 days. Positive cultures were frozen at -20°C until typed.

Identification of Viral Isolates. To reduce the possibility that isolates were mixtures of viruses and would therefore be impossible to type, a single limiting dilution passage was made with each virus.

Ten-fold dilutions of virus ranging from 10^{-3} to 10^{-9} were prepared in maintenance medium and 0.2 ml amounts of each dilution were inoculated into each of two cell cultures of the kind in which the virus originally was isolated. The cultures were incubated at 36°C on a roller drum for five days and one tube from the highest dilution showing CPE was used to inoculate six additional tube cultures. When the CPE in these cultures was 75-100% complete, they were frozen and thawed, clarified by low speed centrifugation, distributed into 1 ml amounts and stored at -20°C. To reduce the costs and labor of typing the viruses isolated from sludge, 96-well, flat bottom tissue culture plates (Limbro) were used to titrate and type all isolates.

To determine the infectivity titers of the isolates, ten-fold dilutions of each virus were prepared in maintenance medium. Using a micropipette, 0.025 ml of each dilution was delivered into each of two wells of a microtiter plate. To each well,40,000 of the appropriate cells in 0.15 ml of growth medium were added. The plates were sealed with pressure sensitive plastic sheets, gently agitated to mix the virus-cell suspensions and incubated at 36°C for six days. Subsequently, the fluids were withdrawn by suction and one drop of formalin-crystal violet (7.5%) stain was added to each well. After 5 minutes, the staining solution was removed by gently washing in tap water and the plates were allowed to air dry before being read. The CPE was visualized by unstained wells where the cells had been destroyed by virus. The titers were determined by the method of Reed and Muench (1938). A calculated test virus dose (TVD) containing 1000 TCD_{50}/0.025 ml was used for attempts to type isolates.

Lim Benyesh-Melnick (LBM) serum pools A-H and J-P obtained from the Research Resources Branch of the National Institute of Allergy and Infectious Diseases (NIAID) were used for typing enteroviral isolates. The procedures used essentially were those recommended by the NIAID in its instructions of April, 1972 that were supplied with the pools. Where necessary, type specific sera also supplied by the NIAID were used to do special tests and to confirm results obtained with the pools. The LBM pools were rehydrated in sterile distilled water, pools A-H were diluted 1:10 and pools J-P were used undiluted. The serum pools were inactivated at 56°C for 30 minutes prior to use.

To a pair of wells on a microtiter plate, 0.025 of a serum pool was added. To each serum pool, 0.025 ml of virus containing 1000 TCD_{50} was added. After gentle mixing, the plates were covered with plastic sheets and incubated for two hours at room temperature. To determine the actual amount of TVD used in the tests, ten-fold dilutions (10^{-1} - 10^{-4}) of each TVD were made at the end of the incubation period and 0.025 ml of each dilution was placed into each of four wells. Subsequently, 40,000 cells in 0.15ml of growth medium were added to each well. Following gentle shaking, the plates were sealed and incubated for six days at 36°C. The plates were stained and read as described above and the viruses were identified with the identification tables provided with the LBM pools.

The reoviruses were presumptively identified on the basis of the kind of CPE produced and by growth only in primary monkey kidney cultures. These isolates were identified as Reoviridae by electronmicroscopy. Briefly, pools prepared from each isolate were partially purified by extraction with fluorocarbon and then centrifuged at 50,000 x g for 60 minutes. The pellets obtained were resuspended in one drop of sterile, pyrogen-free water, one drop of 3% phosphotungstic acid (pH 7.0) was added to the suspension and a drop of the mixture was placed on a 300-mesh, Formvar-carbon-coated copper grid. Excess fluid was removed with filter paper within 10-30 seconds and the grid was allowed to air dry. Grids were examined in a Hitachi HU-12 electron microscope and pictures were taken at magnifications of 70,000 - 100,000. The viruses were identified as Reoviridae by their typical morphology.

Viruses that were not typed because of an inappropriate TVD, were retested with a readjusted TVD. Those isolates that could not be typed, were put through another limiting dilution step to separate possible mixtures of viruses, this time using six tubes per dilution and retested. Thirteen viruses which could not be identified in our hands were finally identified through the courtesy of Ms. Jill Baxa at the Ohio Department of Health Laboratories.

Serum Neutralization Tests. The 23 enteroviruses and the cell culture systems used for serum neutralization tests (SNT's) with human sera are listed in Table 15-24. The viruses were prototype strains obtained from the Research Resources Branch of the NIAID. Although some attempt was made to pick a broad spectrum of serotypes for use in the tests, on a practical basis many of the selections were based on the ability of the viruses to replicate in either RD or BGM cell cultures. It would have been impractical to do large number of SNT's if three or four different kinds of cell cultures and infant mice had to be used for the tests. The viruses were passaged until the infectivity titers obtained in microplates were high enough to allow them to be used in the tests. Care was taken to reduce the possibility of cross contamination of the seed virus pools. Only one virus was handled at a time, the work area was cleaned and exposed to ultra violet irradiation between viruses and lab workers washed their hands and changed outer garments before handling a new virus. The final seed pool of each virus was frozen at -70°C in small aliquots, assayed for infectivity, tested for sterility and typed with reference antisera using at least a viral TVD of 1000.

For neutralization tests, three to four serial sera from one individual were tested simultaneously. The initial dilution for the baseline serum in each test was 1:5. A 1:10 dilution was used for other sera. Thus, baseline sera were tested at dilutions of 1:5-160 and the others at 1:10 - 1:320. Usually three separate tests were needed to test 20-40 sera against all 23 enteroviruses. Sera were heat inactivated at 56°C for 30 minutes, bulk two-fold dilutions were prepared in tubes and a micropipette was used to deliver 0.025 ml amounts to duplicate wells in microtiter plates. A test virus dose for each virus calculated to contain 100 TCD_{50} in 0.025 ml of maintenance medium diluent was added to the appropriate serum dilutions. The plates were agitated gently, covered with plastic sheets and incubated for one hour at room temperature. A 1:2 dilution of each TVD was made and incubated along with the serum-virus mixtures. Approximately 15 minutes before the end of the incubation period, the TVD's were diluted 10^{-1} - 10^{-4} and each dilution was distributed in 0.025 ml amounts into four wells. Micropipette tips were changed between the making and delivery of each ten-fold dilution of the TVD's. Depending on the virus, either 40,000 RD or BGM cells contained in 0.15 ml of growth medium was added to each well. The plates were agitated gently, sealed and incubated at 36°C for six days. The procedures for staining and reading the plates were similar to those described above under Identification of Viral Isolates. The serum titer was the highest dilution of serum that completely inhibited CPE by the TVD in both wells. A four-fold or greater increase in titer between two serial sera tested simultaneously was considered significant. Every significant rise in serum titer was confirmed by at least one repeat test.

Detection of Antibody to Hepatitis A Virus. A radioimmunoassay kit, trademark HAVAB, from the Abbott Laboratories Diagnostic Division was used. Baseline sera obtained from human subjects prior to the application of sludge were tested initially. Those that were definite positives were considered already to have had Hepatitis A viral infections and were not tested again. Individuals who lacked detectable antibody were tested throughout the course of the study to follow them for conversion to positive. The procedures used were those described in the Abbott kit and were followed carefully. Tests giving questionable results or improper control values were repeated.

RESULTS

Viral Isolations from Sludge: the Medina 300 Plant. Sludge samples from this Medina plant yielded the highest percentage of isolates (92%) and most often yielded more than one virus from a sample, a mean of 1.57 viruses per positive sample. Fifty-eight of 63 samples yielded one or more viruses (Table 15-25). Two or more viruses were isolated from 30 of the 63 samples (48%) (Table 15-26). The viruses most commonly associated with multiple isolations were echovirus 7 from 15 of 27 samples (56%), coxsackievirus B3 from 10 of 27 (37%) and poliovirus 2 from 9 of 27 samples (33%). Three samples yielded three viruses. These combinations were echovirus 7, poliovirus 2 and reovirus; coxsackievirus B3, echovirus 11 and poliovirus 2 and coxsackieviruses B2, B3 and echovirus 11. The most frequent viruses isolated were echovirus 7 (28/91, 31%), coxsackievirus B3 (15/91, 16%) and poliovirus 2 (17/91, 19%). Of the 12 different types of viruses isolated, not counting the polioviruses, 4 of the echoviruses and all 3 of the coxsackie viruses were included in the serum neutralization tests used to monitor our subjects for occurrence of enteroviral infections (Table 15-25).

The Medina 500 Plant. The isolation rate from sludge samples from this plant was somewhat lower than that from the 300 plant. See Tables 15-27 and 15-28 for these results. Forty-three of 63 samples (68%) were positive. An average of 1.37 viruses were isolated per positive sample. Fifteen of 63 samples (24%) yielded two or more viruses. Again, echovirus 7 was most frequently found among multiple viral isolates (7/15, 47%). Coxsackievirus B3 was found in 6 of 15 (40%) and polioviruses were associated with 5 (33%) of the 15 samples. One sample yielded three viruses coxsackieviruses B3, B4 and poliovirus type 3. The most frequent viral isolates obtained were similar to those from the M300 plant. These were echovirus 7 (22/59, 37%), coxsackievirus B3 (10/59, 17%) and the polioviruses (12/59, 20%). Of the viruses isolated from the M500 plant, 4 echoviruses and 2 coxsackieviruses were used in the serum neutralization tests to monitor human infections.

The Columbus Plant. Of 123 sludge samples tested, 81 (66%) were positive for 1 or more viruses. Tables 15-29 and 15-30 summarize these results. An average of 1.35 viruses were isolated per sample. Twenty-eight of 123 samples tested (23%) yielded two viruses. Coxsackievirus B3 and echovirus 24 were the most common multiple isolates with eleven of the 28 positive samples (39%) yielding one of these viruses. Five of these 28 samples (18%) yielded polioviruses. Coxsackievirus B3 and echovirus 24 were isolated together from 8 of these (29%) samples. Of the 109 viruses isolated from the 123 samples tested, the most frequent viruses isolated were coxsackievirus B3 (15/109, 14%) poliovirus 2 (15/109, 14%) echovirus 7 (13/109, 12%) and echovirus 24 (13/109, 12%). Of the 109 viruses isolated, 9 echoviruses and 5 coxsackieviruses were included in the serum neutralization tests with human sera.

The Springfield Plant. Twenty-nine of 58 sludge samples tested (50%) were positive for 1 or more viruses, the lowest isolation rate from any of plants in the study. Thirty-eight viruses were isolated from 29 positive samples (1.31 viruses/sample). See Tables 15-31 and 15-32 for these results. Two or more viruses were isolated from 8 (28%) of the 29 positive samples. Coxsackievirus B3 and poliovirus 2 were the most common isolates. Echovirus 7, a common isolate from the other sewage plants, was only isolated twice during the first year of the Springfield study. Coxsackievirus B3 was most commonly found (4 of 8 samples) among the multiple isolates. Ten of the viral serotypes isolated were used in the serum neutralization test to detect enteroviral infections in our human subjects.

Summary of Viral Isolations from all Sludge Samples. Table 15-33 summarizes the frequency of single and multiple viral isolations from all sludge samples. One or more viruses were isolated from 211 (69%) of 307 samples. Of these, 130 yielded one virus, 2 viruses were recovered from 76 samples and 5 samples yielded 3 viruses. Thus, 38% of the positive sludge samples and 26% of all samples tested contained either 2 or 3 viruses that were capable of being isolated by the methods employed. The viruses isolated from all sludge samples are presented in Table 15-34 according to serotype and location. Of the 297 viruses isolated, the most common viruses encountered were echovirus type 7 viruses (65/297, 22%), 47 (16%) were coxsackie B3 viruses, 45 or 15% were poliovirus 2 viruses and 18 (6%) were echovirus type 24 viruses. The number of samples from which viruses were recovered was lowest for the Springfield plant (50%) (see Table 15-31) where the number of viruses isolated per sample tested was only 38 out of 58 or 0.66 viruses per sample. The highest number of viruses isolated per sample was obtained from the Medina 300 plant where 91 viruses were recovered from 63 samples for an average of 1.44 viruses from all samples. Most of the echovirus 7 isolates came from the Medina and Columbus plants. Echovirus type 24 was isolated most often from Columbus sludge whereas coxsackievirus B3 virus was a common isolate from the sludge of all 4 plants. No coxsackievirus B1 or B6 viruses were isolated. Excluding the polioviruses, 22 different enterovirus serotypes were isolated from the sludge of the four sewage plants. Fourteen of these serotypes were included in serum neutralization tests included in the panel used to detect enterovirus infections in our human subjects.

Viral Spectrum of Cell Cultures Used for Isolation from Sludge Samples. Table 15-35 shows all the viruses obtained from sludge according to type and kind of cell culture in which they were isolated. Of 297 viruses isolated, 179 (60%) were recovered in RD cells, 45 (15%) in BGM and 77 (26%) in HeLa cell cultures. For those viruses that were isolated on at least 5 occasions, 7 echoviruses (types 3,6,7,11,20,24 and 30) were recovered almost exclusively in RD cells. Six echovirus serotypes (types 13,15,17, 22,25 and 27) and 2 coxsackievirus A viruses, (A9 and A16), although isolated fewer than 5 times, also were recovered only in RD cell cultures. HeLa cells were useful particularly for isolation of Coxsackievirus B3 and B5 viruses whereas BGM cells were the only ones in which coxsackievirus B2 viruses could be isolated. Ten different sludge samples yielded the same virus in 2 different cell culture systems, poliovirus type 2 twice from each of 8 samples, poliovirus type 3, twice from one and coxsackievirus B3 twice from one sample. Table 15-36 summarizes the cell culture or combination of cell cultures in which these viruses were isolated.

Effects of Season on Viral Isolation Rates from Sludge Samples. Viral isolation rates from sewage sludge obtained from the 4 treatment plants in our study were examined for seasonal effects. Isolation rates for the months of July through October and January through April were compared. For all plants, at least 2 years of data were available for these "cold" and "warm" months. Results from the Medina 300 plant could not be evaluated because the viral isolation rates were consistantly high throughout the course of the study. Table 15-37 gives the results of such a comparison from the Medina 500 plant. With one exception, all sludge samples (94%) collected during the months of July through October yielded viruses. On the other hand, only 44% of samples collected during the months of January through April yielded viruses. The viral isolation rate during warm months was significantly greater, $X^2 = 9.90$, $P = <0.005$. A similar analysis of results with Columbus sludge (Table 15-38) did not show a significant difference in viral isolation rates ($X^2 = 1.17$, $P = <0.3$) between warm and cold months. Table 15-39 shows the viral isolation rates from sludge collected at the Springfield sewage treatment plant during warm and cold months. The isolation rates were significantly different with a Chi square value of 13.05 ($P = <0.0005$). Thus, in 2 of 3 treatment plants where a statistical evaluation could be done, viral isolation rates were higher from sludge collected during warm months. Extending or shifting the months compared by one month in either direction, did not affect the results.

Viruses from Stool Samples. The viruses isolated from stool samples can be seen in Table 15-40. The results are presented according to study status (sludge subjects or controls) and geographic location (source of sludge). The subjects from whom viruses were isolated are identified by their study number. The first 4 numerals of each study number identifies the farm. Thus, subjects where the first 4 numbers of the study number are identical, were either members of the same household, the most common situation or resided on the same farm. Sixteen viruses were isolated from the stools of 15 people residing on sludge-receiving farms. Echovirus type 7 was isolated from 2 different people residing on farm 3007 and from 2 on farm 4004. Nine viruses were isolated from the stools of 9 individuals living on control farms. Echovirus type 26 was isolated from 2 family members residing on farm 3508. Using a matched pair X^2 test (McNemars) (Siegel, 1956A) and paired sludge-receiving and control farms, the results show that there is no significant difference ($X^2 = 1$, $P = <0.32$) in the frequency of any virus being isolated from subjects residing on sludge-receiving and control farms. See Table 15-41.

Serum Neutralization Test Results: Comparison of the Prevalence of Antibody to 23 Enteroviruses Between Baseline Sera from Individuals on Sludge and Control Farms. Although all farms in this study were randomized into sludge and control groups, for additional assurance that both groups were equally susceptible to the viruses used for surveillance, the proportion of susceptible individuals in each group was compared. Baseline (before sludge application) sera were tested at a 1:5 dilution in neutralization tests against the 23 enteroviruses. For our purposes, individuals with serum neutralizing antibody titers of less than 5 for a particular virus were considered to be susceptible to infection with that virus. For each family, the proportion of susceptibles was calculated for each virus. Because of distribution problems, the number of people on each farm varied, the nonparametric Wilcoxen Sign Rank Test was employed to compare the number of susceptibles in each group at the beginning of the study. Table 15-42 shows the results of this analysis. The only significant difference between the number of susceptibles in the two groups was for coxsackievirus B1 where P was <0.05. This finding would be expected by chance alone.

Serum Neutralizing Antibody Rises. All enteroviral infections as detected by 4 fold or greater rises in titer between 2 serially collected sera are presented in Table 15-43. The subject numbers are grouped by family where appropriate, and the serum neutralizing titers given are for the virus indicated. A total of 124 rises (infections) were detected in 67 people. Of these, 69 rises occurred in 34 sludge subjects and 55 in 33 control subjects. Individuals with more than one titer increase during the same time interval and some with several antibody increases over the study period were not uncommon. For example, subject number 100402 had 4-fold or greater rises in neutralizing antibody to 6 different enteroviruses during the period 8-8-78 and 12-5-78. Two 4-fold or greater antibody increases occurred during the period 12-5-78 - 3-13-79 and one occurred between 3-13-79 and 8-7-79. If we ignore the possibility of heterologous antibody responses, during a one year period this subject may have experienced as many as 9 different enterovirus infections. The distribution of 124 antibody rises observed in 67 individuals is shown in Table 15-41. Two or more rises occurred in 24 subjects during the relatively short period of the study. The possibility can not be excluded that some of these multiple rises, particularly those occurring during the same time interval, may have been due to heterologous antibody responses. Table 15-45 shows the neutralizing antibody rises in 67 subjects according to virus. The rises were rather evenly distributed between the coxsackieviruses (51%) and the echoviruses (48%). The viruses for which antibody increases occurred most often were coxsackieviruses B3, B5, A3, A7 and echovirus 25. Coxsackievirus A15 was the only virus of the 23 used in our neutralization tests for which no rises were detected over the course of the study.

Analysis of Neutralizing Antibody Data from Sludge and Control Subjects: Statistical Considerations, Methodology, Results and Discussion. To determine whether individuals on sludge receiving farms experienced a higher rate of antibody rises than individuals on control farms (and because of the timing of blood sample collections) it was necessary to match farms by county and by a 6 month time period following each application of sludge. While it may have been more realistic to limit the period of surveillance after sludge application to 3 months as was done in the analysis of human illness data earlier in this report, it would not have allowed in all cases enough time following sludge application for an infection and a rise in antibody titer to occur. Practical considerations required that blood samples be limited to 3 times a year. Ninety of the 92 farms included in the statistical analysis which follows provided 2 blood samples over the 6 month intervals following applications of sludge. Only one blood sample was obtained from the individuals on 2 farms over the 6 month interval following sludge application, and consequently, the observation period had to be slightly lengthened. In any case, the extension of the period of surveillance to as long as 6 months after the laying of sludge may very well include changes in antibody titer that are not sludge related. However, this bias should be distributed equally in both groups of farms and thus have no effect on any estimate of risk. For all 92 farms, then, any fourfold or greater increase in antibody titer between two successive blood samples was considered as evidence of an infection occurring in the reference period.

The almost 300 individuals living and/or working on the farms in this study did not serve as the units of analysis below. Farms were considered the units of analysis because they may be assumed to be more like each other than were the average individuals in the study. In short, individuals may not be independent. Therefore, special statistical techniques or conceptual considerations are needed for analyzing these data.

In the analysis which follows, the dependent variable or response variable in the statistical sense will be categorical, specifically dichotomous: a fourfold rise in antibody titer by at least one family member or the absence thereof. One straightforward statistical test for the dichotomous response variable often useful in studies of matched pairs is the 2 x 2 contingency table arranged as in McNemar's Test (Siegel, 1956A). However, in the present study, this method appears to have only limited value, as will become apparent. As it was not feasible to match farms on family size or number of weeks between blood samples, these variables would be potential confounders and would have to be treated accordingly. In the matched pair multiple logistic regression used here (and described previously in Section 11) (Tandon et al., 1983; Koch, 1970), these two explanatory variables were included in the model along with the major variable of interest, sludge/control.

Each of the first two sludge applications were analyzed separately, yielding 46 pairs and 29 pairs of farms, respectively. The baseline serum for the first sludge application was taken before sludge was applied. For the analysis of the effect of the second sludge application, the baseline serum was a serum taken before or no more than 15 days after the second application of sludge. It was considered that any antibody rise occurring within that 15 day period probably would not have been due to sludge exposure. (There were too few farms and, therefore, pairs available for analysis from the third sludge period). Finally, an analysis was conducted on all 46 pairs of farms for the entire 3 year study period where the response variable was any rise in antibody titer between two successive blood samples at any time. Although the overall health effects of sewage sludge applied to farm land is the subject of the health portion of this report, this section of the report deals only with relative differences in the number of rises in antibody titer in individuals exposed and unexposed to sludge.

Tables 15-46, 15-47, and 15-48 summarize the results of modeling the health effects of sludge for 46 matched pairs of farms at the time of the first sludge application, 29 pairs at the second application, and finally, for 46 pairs over the entire period between the first laying of sludge (for each pair) and the end of the project. (It should be noted that farm pairs were entered in the study anew throughout the period 1978 to 1981). Each table shows simple and partial regression coefficient and their standard errors for sludge exposure and for the potential confounders.

Tables 15-46 and 15-47 indicate that sludge exposure is not related to differential increases in antibody titer. Logistic coefficients range between .057 and .093 in the 4 analyses summarized in the two tables, associated with odds ratios ranging from 1.06 to 1.10, none even close to significance. Surprisingly, neither of the two confounding variables were apparently capable of biasing the outcome of this study; neither proved to be associated with a fourfold or greater increase in antibody titer. As these two tables correspond to two reference periods separated by approximately one year, the consistent findings are in favor of the null hypothesis and are not easily dismissed.

Since the variables "time between two successive blood samples" and "number of persons in the family", were not associated with rises, it would be useful to look at antibody rises in the matched farm pairs for the first sludge period. Subsequent to the first application of sludge, 33 of the 46 sludge/control pairs responded similarly with respect to changes in antibody titer. In 15 pairs, both farms showed a rise; in 18 pairs, neither showed a rise. Of the remaining discordant 13 pairs of farms, 7 pairs showed a rise in the sludge farm family and no rise in the control farm, while 6 pairs of farms showed a rise in the unexposed farm and no rise in the exposed farm. Arranged as in McNemar's Test, Table 15-49 reflects this experience. Table 15-48 takes a broader look at the health effects of sludge exposure, taking into account the total experience of each cohort between the initial sludge application and the end of the project. The results of these analyses are entirely consistent with the previous tables, supporting the hypothesis of no effect (of sludge).

A study by Northrup and his coworkers (Northrup et al., 1980) that has some relevance to our study investigated the health effects of aerosols formed at an activated sludge plant. This investigation involved the testing of throat and stool samples from 161 people for viruses and bacteria. Excluding the polioviruses, only 14 enteroviruses were isolated from 541 stool samples (3%), a number too small for analysis under the conditions of their study. In addition, 318 subjects provided blood samples at the beginning and at the end of the project, a period of about 8 months (May-December). The study group was composed of 246 middle class families for which exposure indices were derived based on the average concentrations of viable particles and coliform particles at appropriate sampling sites. These workers also used serum neutralization tests to monitor for infections but this was done with only 12 enteroviruses. These were polioviruses 1-3, coxsackieviruses B1-B5 and echoviruses 3,6,9 and 12. Unfortunately, no attempt was made to demonstrate the presence of viruses in the aerosols emitted from the sewage plant. The frequency of viral infections based on serologic rises was analyzed according to the mean total viable particle exposure indices associated with the serological group. These authors concluded that the risk of infection was not affected by increased exposure to total viable particles emitted from an activated sludge plant.

Frequency of Neutralizing Antibody to 23 Enteroviruses in Human Sera. The percentages of human sera (all ages) with neutralizing antibody to the 23 enteroviruses were tabulated according to virus and are presented in Figure 15-3. A total of 262 sera were tested at a 1:5 dilution. The most prevalent neutralizing antibodies were to Coxsackieviruses (C) A3 and B4 and echoviruses (E) 9 and 25. Almost all individuals tested had antibody for E9. The same kind of information is presented for the coxsackie A, coxsackie B and the echoviruses according to age in Figures 15-4, 5 and 6 respectively. Table 15-50 is a composite of the data from Figures 15-4, 5 and 6. Antibody for E9 appears to be acquired at an early age. All subjects in the 5-12 and 13-21 year age groups had neutralizing antibody for this virus (Figure 15-6). In general, and as one might expect, the number of subjects with antibody increases with age. However, there are some exceptions to this expectation. As can be seen from Figure 15-4, the incidence of antibody for CB1, B2 and B5 was relatively uniform for all 5 age groups. The mean percentage of antibody for the 6 coxsackie B viruses ranged from a low of 22% for the 5-12 year age group to about 30% for the three oldest age groups. The results for the coxsackie A viruses (Figure 15-5) followed a similar pattern with the mean percentages going from 11-32%. The frequency of antibody for coxsackieviruses B6, A11, and A15 was essentially zero. The incidence of antibody for E3 and E6 was relatively uniform for 4 of the 5 age groups but with about a 2 fold lower incidence in the 13-21 age group. The frequency of antibody for E11, 12, 19 and 20 appears to be comparatively low in the 13-21 year age group with the incidence for E20 remaining constant at the higher age groups. Antibody frequency for E7 was at 7% and 11% in the 13-21 and 22-40 age groups, respectively. Overall, the frequency of antibody for 6 of the echoviruses was relatively high in the 5-12 year group, then dipped to low levels in the 13-21 year age group and increased again in the older groups.

Surveillance for Serologic Conversion to Hepatitis A Virus. Since this virus causes an enteric infection, is spread primarily by the fecal-oral route and is a relatively stable virus, it would be expected, like the enteroviruses, to be present in sewage and to survive sewage treatment. In addition, a sensitive, radioimmunoassay was available for detecting antibody to hepatitis A virus. For these reasons, all the subjects in the study were followed for serologic conversion to determine if any hepatitis A infections had occurred during the course of the study. No serologic conversions were observed. None of the individuals negative for hepatitis A antibody at the beginning of the study became positive. Figure 15-4 presents the frequency of hepatitis A antibody according to age. As can be seen from the figure, almost no individuals in the 6-15 year age group had antibody. About 19% of those in the 16-25 year group had antibody. The numbers in these 2 age groups unfortunately are small but when considered with the results in the 26-40 year group (10% positive), together they indicate that antibody for hepatitis A virus is acquired relatively late in this population group. Less than half of the 41-59 year olds had antibody whereas 85% of individuals over 60 years of age were positive.

Spread of Virus in Households With One or More Susceptible Individuals. There were 24 households in which one or more antibody rises were detected and which contained at least one susceptible person (based on no serum antibody at a 1:5 dilution). Please see Table 15-51. The antibody titers and serologic rises shown were detected in the same or the next immediate blood sampling period. The mean number of household members was 3.2 with a low of 2 and a high of 6 individuals per household. The patterns of enteroviral infections detected included households in which every susceptible individual apparently was infected (household 4) with coxsackievirus B3 and echovirus 24 to households 7,12,17,19,21 and 23 where none of the susceptible individuals (excluding the index cases) was infected after introduction of a variety of other enteroviruses. In some cases, preexisting serum neutralizing antibody apparently was not protective, such as CB5 in households 2, 4 and 6, CB4 in household 3, and CB1, CB2, E11, E12 and E24 in household 6. Excluding index cases, there were 77 susceptible people in the 24 households. Of these, 15 (19%) were infected. Of 46 subjects susceptible to coxsackievirus infections, 8 (17%) were infected; of 31 susceptible to echovirus infection, 7 or 23% were infected.

DISCUSSION

Viral Isolations from Sludge. A total of 297 enteroviruses were isolated from 307 sludge samples from all sources. Of these samples, 211 (69%) were positive for one or more viruses. The highest frequency of viral isolations including multiple isolates from single samples was obtained from the Medina 300 plant. Of 63 samples tested, 58 (92%) were positive (Table 15-25) and 30 of the 58 (52%) yielded 2 or more viruses. See Table 15-26. There is no ready explanation for the results from this plant. The materials and methods used for the isolation of viruses from sludge remained constant throughout the course of the study. Both Medina plants are aerobic; the Columbus and Springfield plants use anaerobic methods. The Columbus sludge is dewatered whereas the other treatment plants produce liquid sludge. Relatively little is known about the comparative ability of aerobic and anaerobic treatment for the inactivation of viruses. It has been suggested that aerobic treatment is not as efficient as anaerobic for this purpose (Bitton, 1980). The M300 plant served a rural area with a population equivalent (calculated from biological oxygen demand [BOD] of 5,971 and no industry while the M500 plant served a rural area with a population equivalent of 26,801 and some industry (2 small industrial parks). Two years after initiation of the study (1980), the city of Medina (15,268 people) was tied into the M500 plant bringing the total population served to about 40,900 with some additional industry (to 6.7% industrial). The BOD population equivalent then rose to 66,180. There were no striking differences between the viruses isolated during the first and third year of the study before and after Medina was tied into the M500 plant. See Table 15-27.

Chapter 1 of this report describes the characteristics of the sludges produced by the various treatment plants. With the possible exception of the average solids content, 3.7% for the M300 plant and 2.1% for the M500, there were no apparent differences between the two plants during the first 2 years of the study. It is well established that viruses in sludge are associated primarily with the solids (Clark et al., 1961; Kelly et al., 1961; Moore et al., 1975; Schaub and Sagik, 1975 and Balluz et al., 1977). However, there is no quantitative information available on the possible relationship between the amount of solids in sludge and the presence of viruses. There is therefore no ready explanation for the large difference in viral isolation rates between the two Medina plants.

There are many methods that have been described for isolating viruses from sludge. These range from simply shaking, clarifying and filtering a sample prior to inoculation into cell cultures (Irving and Smith, 1981) to various means of viral elution, extraction and concentration prior to inoculation (Sattar and Westwood, 1976; Landry et al. 1978; Farrah et al., 1981; Berman et al., 1981; Brashear and Ward, 1982; Farrah, 1982). There are no standardized methods available for the isolation of viruses from wastewaters. No longitudinal, comprehensive studies have been done comparing methods of isolation using sludge or sewage samples of varying physicochemical composition, from different geographic areas, and from different populations and types of treatment plants. Comparative studies have been done only with seeded sludges using a few enteroviruses. Others have been done with only one kind of cell culture or with only one sludge sample. It is clear therefore that comparisons of the efficacy of different viral isolation methods from wastewaters are not presently possible.

Some of the comparative or longitudinal studies that have been done indicate that important factors involved in the successful isolation of viruses from wastewaters include the types of cell cultures used, the volume of sample inoculated into cell cultures, concentration methods and whether cell cultures are maintained under fluid medium or an agar overlay. Schmidt et al., (1978), used 101 secondary effluent samples from a single treatment plant to determine the sensitivity of 5 different cell culture systems for the isolation of viruses from wastewaters. Most of the samples had been concentrated either by various filter adsorption-elution procedures or by flocculation. The cell systems used were primary rhesus kidney, a fetal rhesus monkey kidney line, BGM cells, a human fetal diploid kidney line and the RD line of human rhabdomyosarcoma cells. In addition, 3 overlay media were used and plaquing was done both in airtight bottles and in plates incubated in a CO_2 incubator. Using 5 different cell culture systems and partially concentrated samples, Schmidt and her coworkers isolated 1 or more viruses from 61 of the 101 samples (60%). Multiple isolates were common with 27 of the 61 positive samples (44%) yielding 2 or more viruses with some yielding as many as 6 or 7 different viruses. Plaquing was not found to be an efficient means of isolating enteric viruses especially for echoviruses and reoviruses.

The results of Schmidt's study also emphasize the importance of using a variety of cell cultures and inoculating at least duplicate cell cultures with each sample. The BGM line of continuous African green monkey kidney cells was not useful for recovering echoviruses or reoviruses but was sensitive for isolating polioviruses and coxsackie B viruses. The RD cell line was useful for isolating a relatively wide variety of enteroviruses, including some types that previously had not been recovered in continuous cell lines. Primary rhesus kidney cells were necessary for recovery of reoviruses and certain echoviruses, especially types 1,7, 8 and 14.

Under the conditions of our study, 301 viruses were isolated from 307 sludge samples in the three cell lines (RD, BGM and HeLa) used throughout the study. Of these, the RD line was by far the most useful especially for the isolation of the 16 echoviruses from sludge samples. Essentially all the echoviruses were isolated in RD cells. Echoviruses 3, 6, 13, 15, 17, 20, 22, 30 and coxsackieviruses A9 and A16 were recovered only in RD cells. The RD line also appeared to be useful for the recovery of polioviruses. See tables 15-35 and 15-36. Moreover, 64 of 65 echovirus 7 and 10 of 11 echovirus 11 viruses were isolated in RD cells. Our findings with echovirus types 7 and 11 contrast with those of Schmidt and her coworkers who reported that these viruses were not isolated in RD cells (Schmidt et al., 1978). These results may have been due to differences in the sources and processing of sludge samples. In addition, our RD cells were maintained on a medium consisting of Eagle's minimal essential medium in Earle's salt solution containing only 12 ml of 7.5% $NaHCO_3$ per liter as opposed to Liebovitz medium no. 15 used by Schmidt and her colleagues (Schmidt et al., 1975).

In another study (Irving and Smith, 1981) involving a one-year survey of enteroviruses, adenoviruses and reoviruses in effluent from an activated-sludge treatment plant, no special methods for elution and concentration of viruses were used. Samples were shaken for 30 min at 4C and filtered to remove bacteria and fungi prior to inoculation into 20 tubes of each of 4 cell types. Thus an unusually high number (80) of cultures was inoculated with a total of 68 ml of each sample. In addition, HEPES buffer was used in the maintenance medium; the cultures were incubated on a roller drum and refed with fresh maintenance media every 3 to 4 days. The large number of cultures inoculated with each sample and the repeated refeeding of cultures were done because these workers were trying to estimate virus concentration by a most probable-number method. Normally, these methods would be considered heroic if used just for the isolation of viruses from field samples. The cell cultures used were primary cynomolgous monkey kidney, BGM, HeLa-R (similar to our HeLa-M) and Barrie, another continuous human epithelial cell line. These authors state that 750 adenoviruses, 1,237 enteroviruses and 1,258 reoviruses were isolated from about 200 samples of primary and secondary effluent and raw sewage samples.

It can not be discerned from the results what constituted a virus isolation. From the large number of isolates reported, it appears as though every tube culture positive for CPE was considered an isolation. At any rate, adenoviruses and enteroviruses were isolated most frequently from HeLa cells, 85% and 42% of all isolates, respectively. Of the reoviruses, 64% of the isolates were obtained in cynomolgous monkey kidney cells. Although 80% of the enterovirus isolates were typed, only 7 different echoviruses were isolated and no coxsackie A viruses were recovered. However, since this was just a one year study, the relatively small variety of serotypes recovered, may have been a reflection of the viruses present in their community of about 1,000,000 people. Unfortunately, the information on the sensitivity of different cells for primary isolation did not include viral serotypes. To the best of our knowledge, Irving and Smith's report is the only one in which such large numbers of adenoviruses were isolated from effluent samples. This high isolation rate probably was due to the 20 HeLa cell cultures inoculated with each specimen, refeeding the cultures at frequent intervals (every 3 to 4 days) and the 3 to 4 week observation period for the appearance of adenovirus CPE. These workers did not describe what precautions were used to reduce viral cross contamination of cultures during the extensive and frequent refeeding procedures that must have been required by their methodology.

In temperate climes, it is well established that enteroviral infections peak during the summer and fall seasons of the year. It is therefore not surprising that the highest frequency of isolation and levels of enteric viruses in raw and treated sewage have been detected during this time (Melnick et al., 1950; Bloom et al., 1959; Horstmann et al., 1973; Irving and Smith, 1981; Sellwood et al., 1981; Goddart et al., 1981). In our study, the seasonal effects on viral isolation rates could not be evaluated for the Medina 300 plant since almost all the samples yielded one or more viruses regardless of the season. For the Springfield and Medina 500 plants, the isolation rates were significantly higher during the months of July through October. This was not true for the Columbus plant. See Tables 15-37, 15-38 and 15-39. The Columbus plant serves a much larger population than the others and perhaps enteroviruses are being shed into sewage throughout the year. Further, sludge produced in the Columbus plant is dewatered by centrifugation and the high solids content may have increased our ability to recover viruses even if initially present in small numbers in sewage entering the plant. Quantitation of infectious virus in sludge samples obtained throughout the year might have helped answer this question.

The results of this part of our study reemphasize the importance of using more than one kind of cell culture for the isolation of viruses (Lee et al., 1965; Schmidt et al., 1978; Melnick, 1979; Irving and Smith, 1981). For best results, cell cultures known to be sensitive to the widest possible spectrum of enteric viruses should be used. The number of each kind of cell culture inoculated also appears to be important especially if viruses are present in small amounts. The isolation of adenoviruses and reoviruses appears to be related to the length of observation, with cell cultures often developing cytopathic effects 10 to 25 days post inoculation.

It is not unusual to recover more than one virus from a single wastewater sample. Schmidt and her colleagues using 4 different cell cultures for virus isolations, reported the isolation of 2 or more viruses from 27 of 61 (44%) positive influent samples with one sample yielding as many as 7 different viruses (Schmidt et al., 1978). In a study by Sellwood and her coworkers, where 5 different cell cultures were used, 75% of inlet and 36% of effluent samples yielded 2 or more different enteric viruses (Sellwood et al., 1981).

In the present study, 81 of 307 sludge samples tested (26%) or 38% of the 211 positive samples yielded 2 or more enteroviruses when 3 different cell cultures were used. Clearly, the results of any study of the utility of cell cultures for the recovery of viruses from wastewaters are influenced by many factors. These include the kind of treatment, if any, to elute and concentrate viruses, the stability of viruses under various environmental conditions, the length of the replication cycle of the viruses, and on the epidemiology of enteric viruses in the community(s) that forms the wastewater being tested.

Viral Isolations from Stools. Monitoring human stool samples for viruses was not helpful in trying to determine if exposure to sludge increased the risk of enterovirus infections. The frequency of virus isolations was too low and the results were confounded by multiple isolations among family members (see Table 15-40). We obtained stool samples only 3 times each year and although this may have been adequate for detection of parasitic infections, it probably was not adequate sampling especially for detecting echoviruses which might be shed in feces for less than 2 weeks following infection (Kogan et al., 1969). In addition, Kogan and colleagues have shown that failure to test respiratory secretions along with fecal samples for enteroviruses would have caused them to miss about 23% of coxsackievirus and 32% of echovirus infections. These investigators also reported that 8 coxsackievirus infections missed by viral isolation methodology were detected by serum neutralization tests with appropriately spaced serum samples. More closely spaced sampling of stool and respiratory samples probably would have been another accurate method of monitoring for enterovirus infections.

However, the conditions of our study with its many facets, limited resources and the logistics of obtaining and testing samples every 2 weeks from a scattered population of rural subjects, would have made it extremely difficult to monitor infections using stool samples and respiratory secretions.

Serum Neutralization Tests: Detection of Enterovirus Infections. The serum neutralization test is sensitive, accurate and for our purposes has been a practical means for detecting enterovirus infections in humans. For detecting antibody to enteroviruses, the neutralization test is recognized as accurate and virus specific (Melnick, 1982). However, with the exception of the polioviruses, little information is available about heterotypic neutralizing antibody increases in humans following enterovirus infections. The recognized serologic cross reactions among coxsackieviruses and echoviruses as determined with hyperimmune animal sera have been summarized by Melnick (Melnick, 1979). Cross reactions have been detected between coxsackieviruses A3 and A8, A11 and A15, and A13 and A18. Serologic relationships also have been found between echoviruses 1 and 8, 12 and 29, and 6 and 30. Of these viruses, coxsackieviruses A3, A11 and A15 and echoviruses 6 and 12 were included in our neutralization tests. Coxsackievirus A8 which crosses with A1 was not included and although 2 rises were detected for the A11 virus, no rises were observed with A15 which is related to A11 according to tests with hyperimmune animal sera. The results with animal sera, however, may not be relevant to the human situation where considerable prior experience with a variety of enteroviruses may be the rule, increasing the possibility of heterologous antibody rises following an enterovirus infection.

We monitored for infections with 23 of about 70 enteroviruses by serum neutralization tests. Although this was an adequate sampling, we undoubtedly missed some infections caused by other enteroviruses. We did detect a total of 124 neutralizing antibody rises in 67 subjects, 69 rises in 34 sludge subjects and 55 in 33 control subjects. See Tables 15-44 and 15-45.

Incidence of Neutralizing Antibody in Human Sera to 23 Enteroviruses. With the exception of coxsackieviruses B1 and B5, the frequency of antibody to the 23 enteroviruses generally increases with age. See Table 15-50 and Figures 15-4, 5 and 6. The absence of antibody in our cohort for the B6, CA11, CA15 viruses and the low frequency of antibody for E26 is noteworthy. These results suggest that these viruses have not been active recently in this population. On the other hand, almost all individuals had antibody for E9 virus and the frequency of antibody for the CB4, CA3, E11 and E25 viruses was relatively high. If we ignore the possibility of heterologous antibody responses to related viruses as an explanation for the high incidence of antibody to echovirus 9, we then must conclude either that this virus is constantly endemic in our cohort or that infections occur very early in life and that antibody persists into old age (Figure 15-6). The apparent decrease in the frequency of antibody in the 13-21 year age group for some of the echoviruses e.g., E3, 6, 11, 12, 19 and 20 (Table 15-50, Figure 15-6) may not be real and could be due to the small numbers in the 5-12 and 13-21 year age groups.

In another study where sera from 308 individuals ranging in age from 6 to over 60 years, were tested for antibody against echovirus 9 (Northrop et al., 1980), only 29% contained antibody. We have no ready explanation for these differences. Perhaps demographic differences might have played a role. Our population consisted of farmers and people who live on farms, whereas the population studied by Northrop and his colleagues was in the vicinity of a sewage plant in the suburbs of Chicago (Skokie). In addition, no methods were described for the serodiagnosis of enteroviral infections.

Incidence of Hepatitis A Antibody According to Age. It is now well recognized that the prevalence of antibody to hepatitis A virus increases with age (Kashiwagi et al., 1983; Briem et al., 1982; Szmuness et al., 1976; Frosner et al., 1979; Szmuness et al., 1977; Hawkes et al., 1981; Burke et al., 1981; Moritsugu et al., 1978). There also is evidence that lower socioeconomic groups especially where poor hygienic standards exist, acquire antibody to hepatitis A virus earlier in life (Cherubin et al., 1978; Dienstag et al., 1978). Dienstag and his coworkers hypothesized that where sanitation and hygienic practices have improved, there has been a decline in hepatitis infections in the younger age groups and that the higher frequency of antibody in older individuals is a reflection of the higher infection rates that were prevalent when they were young. The data on hepatitis A antibody in our cohort appears to support that concept. See Figure 15-7. Of 125 individuals less than 41 years of age, only 10% were positive for hepatitis A antibody. On the other hand, 42% of people 41-59 years of age and 85% of individuals over 60 years of age were positive. If the two oldest age groups are combined, 59% of individuals over 41 years of age have experienced infection with hepatitis A virus.

Viral Infections Among Household Members. The report of Kogan and his colleagues (Kogan et al., 1969) presents their data and discusses the observations of others about the spread of enteroviruses among family members. The results of almost all of these studies are based primarily on viral excretion in feces and not on serologic surveillance which would be important to determine the immune status of household members. Because of the large number of recognized enteroviruses (over 70), the differences in infectivity of different serotypes and even strains of the same serotype, their different host cell spectra for isolation from clinical materials, and the variable duration and rates of excretion in infected individuals, it is difficult to obtain precise data on the spread of enteroviruses within households. Kogan and his coworkers (1969) were able to follow 60 households in which coxsackieviruses caused infections and 28 in which echoviruses were introduced. Since sera were available to follow the appearance of antibody, these investigators were able to follow intrafamilial spread by monitoring viral excretion and the appearance of neutralizing antibody. Their results indicate that the coxsackieviruses infected about 75% and the echoviruses about 40% of all susceptibles.

In our study, there were 24 households containing one or more susceptible members into which an enterovirus was introduced. Since there are many factors that might be involved in the spread of enteroviruses within a household, it is difficult to draw accurate conclusions from our serologic data. Some of these factors include the possibility of heterologous antibody increases, infectivity of the virus, the amount of virus ingested, personal hygiene, intimacy of contact among members of the household, and the possibility that the additional infections observed were acquired from an extra household source. If we ignore the possibility of heterologous rises and assume that the index case was the source of infection within each household, 25% of susceptible individuals acquired infections in the household environment (Table 15-51). Eight of 46 susceptibles (17%) acquired infections with coxsackieviruses and 7 of 31 susceptibles (23%) were infected with echoviruses. Household 4 is noteworthy in that all the susceptible members were infected with coxsackievirus B3 and echovirus 24. Perhaps these viruses are highly infectious. However, we can not exclude the possibility that hygienic practices, degree of intimacy or the presence of young children might have played a role. In contrast, none of 12 susceptibles acquired infections with coxsackievirus B5. In this case, most of the rises occurred in the presence of preexisting antibody which might have influenced the amount and duration of viral excretion. Again, some of these rises might have been heterologous in nature.

Excluding the polioviruses, there is little or no information available in humans about heterologous antibody responses following an enteroviral infection. Schmidt and her coworkers did study antibody responses in monkeys sequentially infected with coxsackieviruses A9, B1 and B3-B6. Heterologous neutralizing antibodies were detected following infections, but only to serotypes with which the animals had experienced a previous infection (Schmidt et al., 1965). In general, the heterologous responses were low and often transient.

Finally, it must be reiterated that our results are based solely on serologic surveillance and therefore may not be comparable to observations which include excretion of virus. Clearly, the most accurate means of obtaining information on enteroviral infections within households would be to monitor infections by following both viral excretion and antibody status at appropriately spaced intervals.

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Figure 1

Flow chart detailing procedure for isolation of enteric pathogens

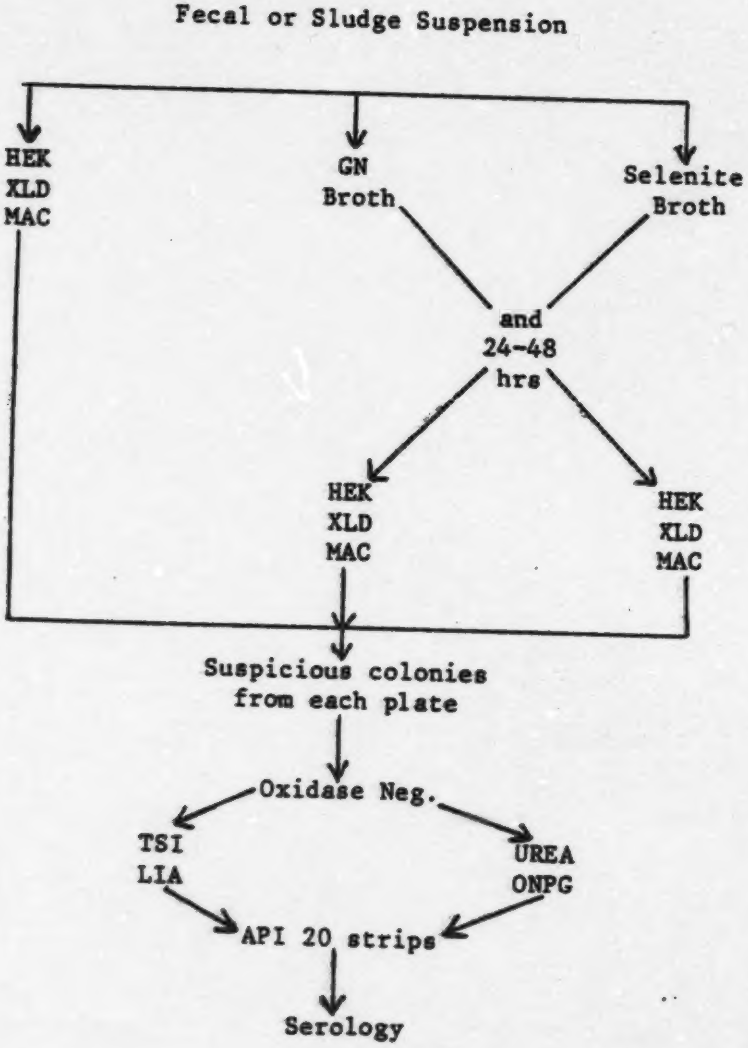
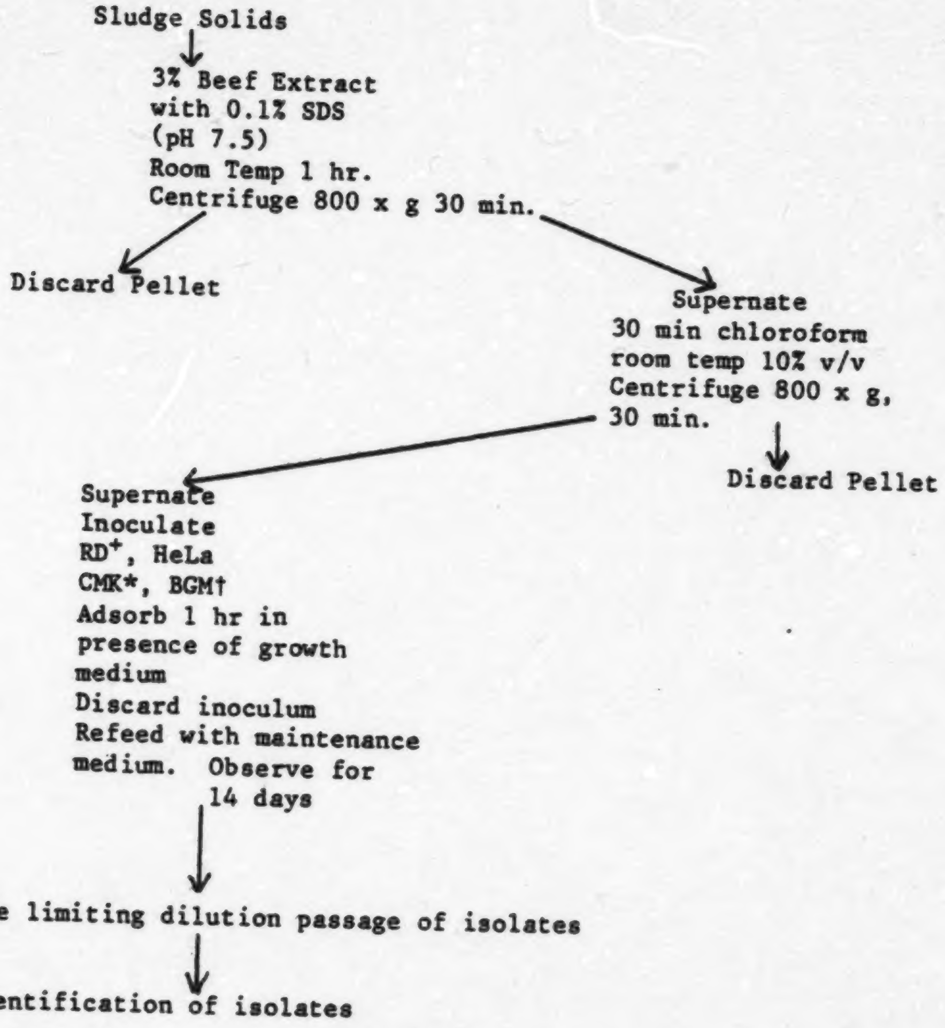

Fecal or Sludge Suspension
HEK
XLD
MAC
GN
Broth
Selenite
Broth
and
24-48
hrs
HEK
XLD
MAC
HEK
XLD
MAC
Suspicious colonies
from each plate
Oxidase Neg.
TSI
LIA
UREA
ONPG
API 20 strips
Serology

FIGURE 15-2

Isolation of Viruses From Sludge


Sludge Solids
3% Beef Extract
with 0.1% SDS
(pH 7.5)
Room Temp 1 hr.
Centrifuge 800 x g 30 min.
Discard Pellet
Supernate
30 min chloroform
room temp 10% v/v
Centrifuge 800 x g,
30 min.
Discard Pellet
Supernate
Inoculate
RD+, HeLa
CMK*, BGM†
Adsorb 1 hr in
presence of growth
medium
Discard inoculum
Refeed with maintenance
medium. Observe for
14 days
One limiting dilution passage of isolates
Identification of isolates

+ RD = rhabdomyosarcoma cell line

* Primary CMK cultures were used to test only 90/307 sludge samples

† BGM = cell line derived from green monkey kidney cells.

Figure 15-3

Percentages of Human Sera with Neutralizing Antibody to 23 Enteroviruses. N=262 Sera Tested at a 1:5 Dilution

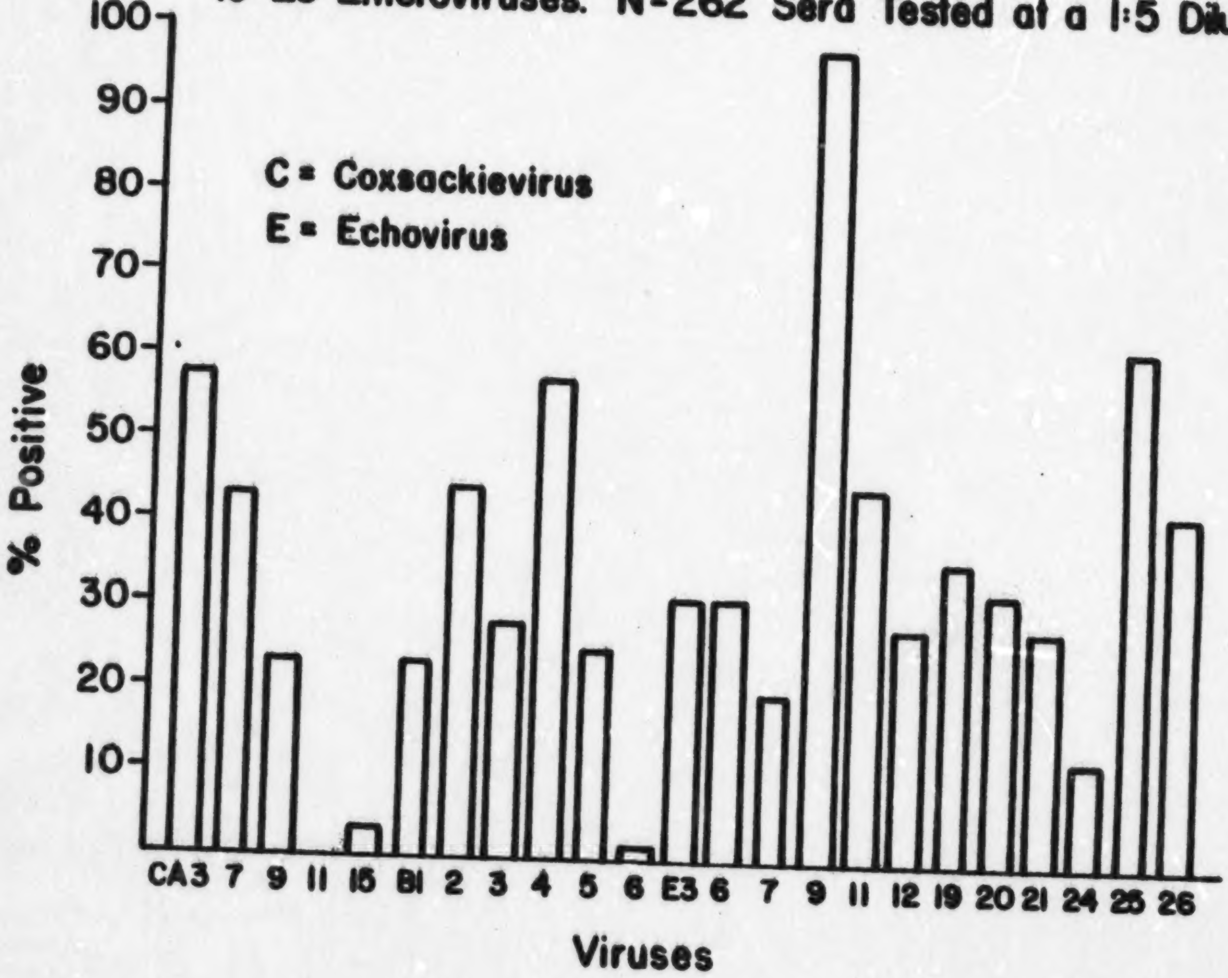


Figure 15-4

Percentages of Human Sera with Neutralizing Antibody to 6 Coxsackie B Viruses According to Age

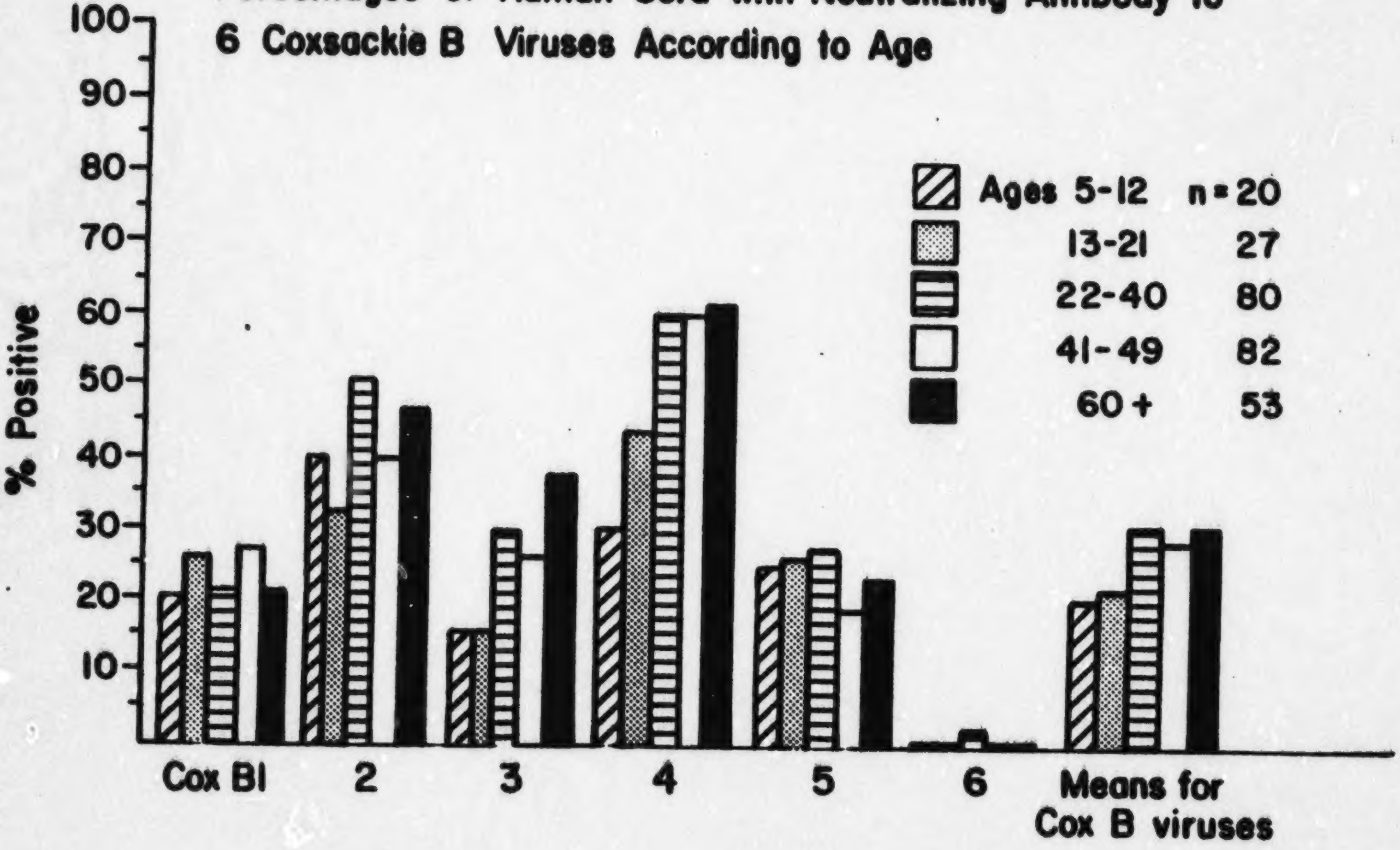


Figure 15-5

Percentages of Human Sera with Neutralizing Antibody to 5 Coxsackie A Viruses According to Age

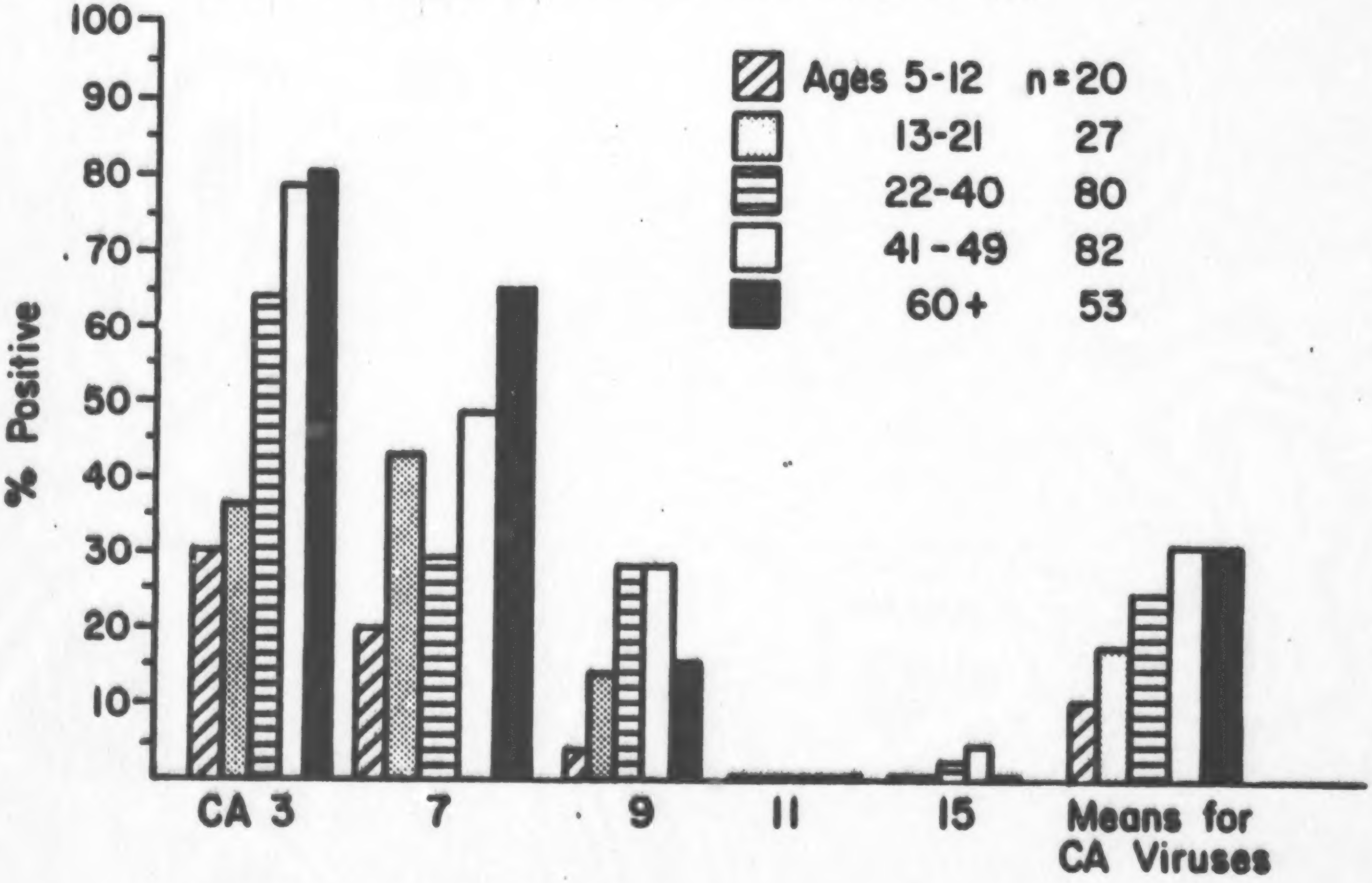


Figure 15-6

Percentages of Human Sera with Neutralizing Antibody to 12 Echoviruses According to Age

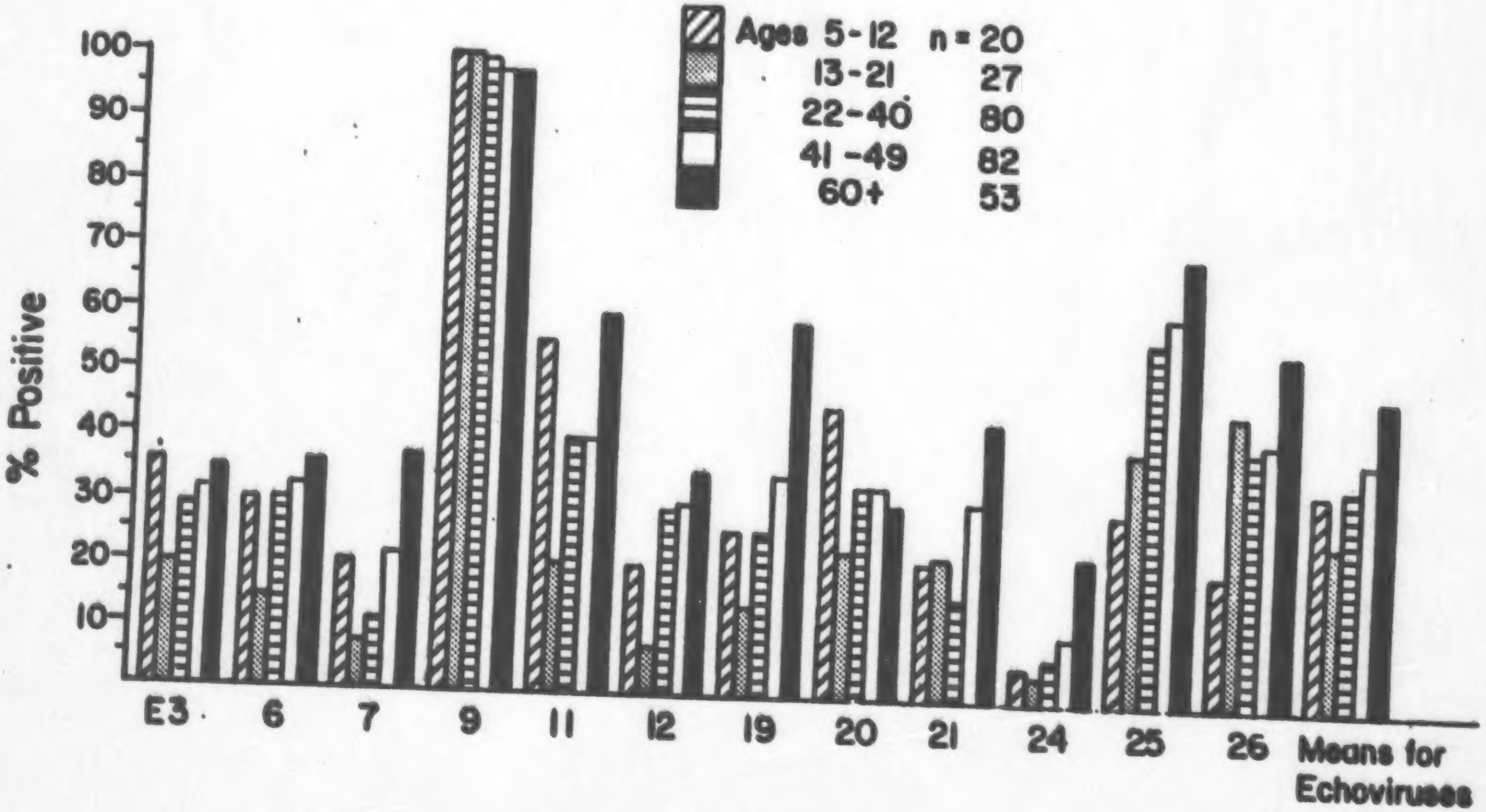


Figure 15-7

Incidence of Hepatitis A Antibody in a Farm Population According to Age (N=261)

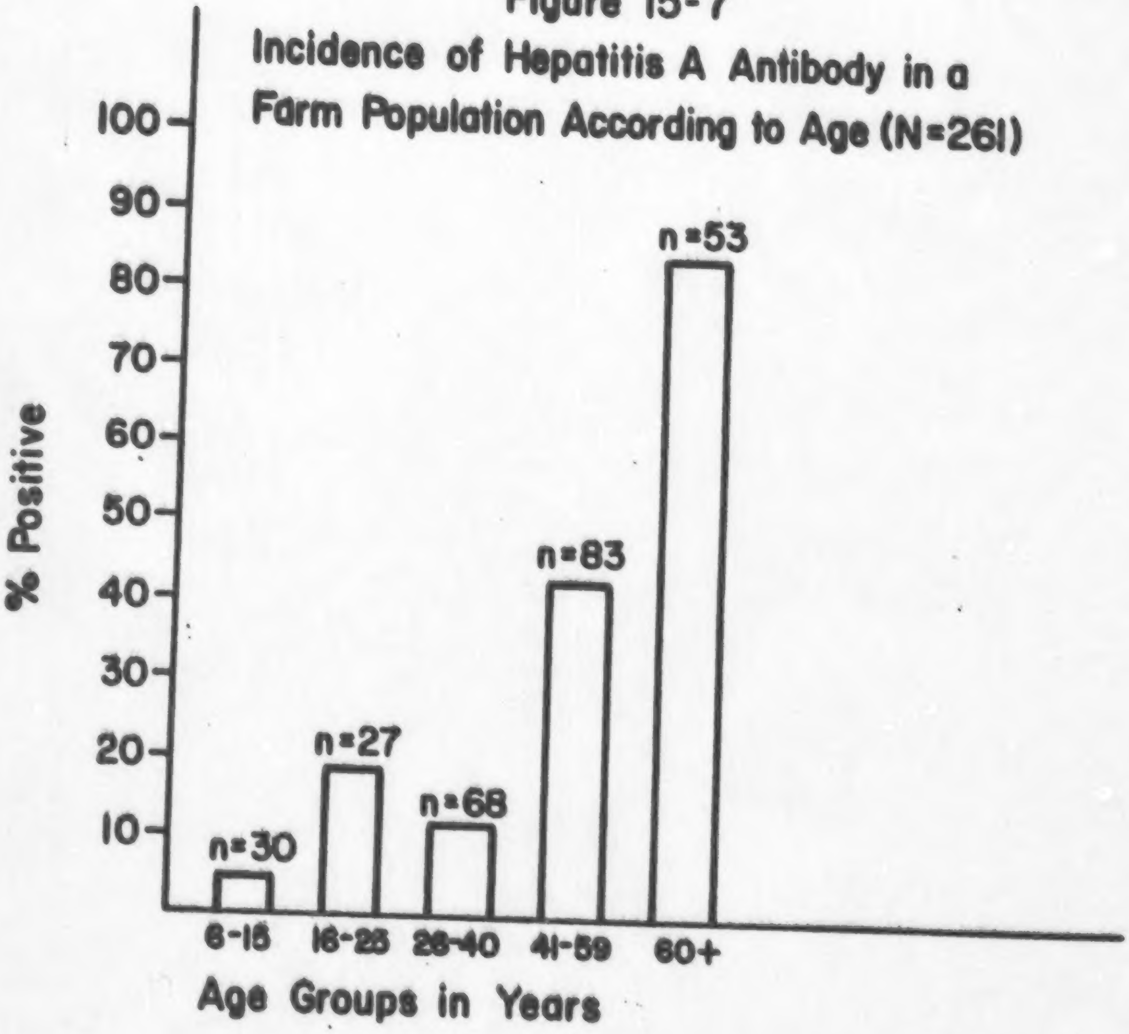


TABLE 15-1

Salmonella Recoveries - Seeded Sludge from Columbus Plant

Tube #	Organism Added Final CFU/ml	24 hrs HE[a]	MAC	XLD	HE[b]	MAC[b]	XLD[b]	7 days HE	MAC	XLD	HE[b]	MAC[b]	XL
1	0	-[c]	-	-	-/-	-/-	-/-	-	-	-	-/-	-/-	-/-
2	1.1 x 10	-	+[d]	-	+/+	+/-	-/+	-	-	-	+/+	+/-	+/+
3		-	-	-	+/+	+/-	+/+	-	-	-	+/+	+/-	-/-
4	1.1 x 10^2	-	-	-	+/-	+/-	+/+	-	-	-	+/+	+/-	-/-
5		-	-	-	+/+	+/-	-/+	-	-	-	+/+	+/-	+/-

Appropriate numbers of organisms were added to 10 ml of sludge (in duplicate), incubated for 24 hours and 7 days at 4°C and isolations were done according to the protocol.

[a] HE-Hektoen Agar; MAC-MacConkey's Agar; XLD-xylose-lysine-deoxycholate agar

[b] From enrichment broth Selenite/GN broth

c = No salmonellae recoverable

d = Recoverable salmonellae

TABLE 15-2

Salmonella Recovery - Seeded Sludge from Columbus Plant

Tube #	Organism Added Final CFU/ml	24 hrs						7 days					
		HE[a]	MAC	XLD	HE[b]	MAC[b]	XLD[b]	HE	MAC	XLD	HE[b]	MAC[b]	XL
1	0	-[c]	-	-	-/-	-/-	-/-	-	-	-	-/-	-/-	-/-
2	2.8 x 10	-	-	-	+/+[d]	-/+	+/-	-	-	-	+/+	-/-	+/-
3		-	-	-	+/+	-/-	+/-	-	-	-	+/-	-/-	-/-
4	2.8 x 10^2	+	-	-	+/+	-/+	+/+	-	-	-	+/+	+/-	+/-
5		+	-	-	+/+	+/-	+/-	-	-	-	+/+	+/-	+/-
6	2.8 x 10^3	+	-	-	+/+	-/-	+/+	-	-	-	+/+	+/-	+/+
7		+	-	-	+/+	-/-	+/+	-	-	-	+/+	+/+	+/-

Appropriate numbers of organisms were added to 10 ml of sludge (in duplicate), incubated for 24 hours and 7 days at 4°C and isolations were done according to the protocol.

[a] HE-Hektoen Agar: MAC-MacConkey's Agar: XLD-xylose-lysine-deoxycholate agar

[b] From enrichment broth Selenite/GN broth

[c] = No salmonellae recoverable

[d] = Recoverable salmonellae

TABLE 15-3

Salmonella Recovery - Seeded Sludge From Medina 500 Plant

Tube #	Organism Added Final CFU/ml	24 hrs						7 days					
		HE[a]	MAC	XLD	HE[b]	MAC[b]	XLD[b]	HE	MAC	XLD	HE[b]	MAC[b]	XLD
1	0	-[c]	-	-	-/-	-/-	-/-	-	-	-	-/-	-/-	-/-
2	2.7 x 10	-	-	-	+/+[d]	-/+	+/-	-	-	-	+/+	-/-	+/-
3		-	-	-	+/+	ND	+/+	-	-	-	+/+	-/-	+/-
4	2.7 x 10^2	-	-	-	+/-	ND	+/+	-	-	-	+/+	+/-	-/-
5		+	-	-	+/+	ND	-/+	-	-	-	+/+	+/-	+/-
6	2.7 x 10^3	+	-	-	+/+	ND	+/+	-	-	-	+/+	+/+	+/+
7		+	-	-	+/+	ND	+/+	-	-	-	+/+	+/+	+/-

Appropriate numbers of organisms were added to 10 ml of sludge (in duplicate), incubated for 24 hours and 7 days at 4°C and isolations were done according to the protocol.

[a] HE-Hektoen Agar: MAC-MacConkey's Agar: XLD-xylose-lysine-deoxycholate agar

[b] From enrichment broth Selenite/GN broth

c = No salmonellae recoverable

d = Recoverable salmonellae

TABLE 15-4

Salmonella Recovery - Seeded Sludge from Springfield Plant

Tube #	Organism Added Final CFU/ml	24 hrs HE[a]	24 hrs MAC	24 hrs XLD	24 hrs HE[b]	24 hrs MAC[b]	24 hrs XLD[b]	7 days HE	7 days MAC	7 days XLD	7 days HE[b]	7 days MAC[b]	7 days XL
1	0	-[c]	-	-	-/-	-/-	-/-	-	-	-	-/-	-/-	-/-
2	1.1 x 10	+[d]	-	-	+/+	+/-	+/+	-	-	-	+/-	+/-	-/-
3		-	-	-	+/+	+/-	+/-	-	-	-	-/-	-/-	-/-
4	1.1 x 10^2	-	-	-	+/+	+/-	+/-	-	-	-	+/+	+/-	+/-
5		-	-	-	+/+	+/-	+/-	-	-	-	+/+	+/-	+/-

Appropriate numbers of organisms were added to 10 ml of sludge (in duplicate), incubated for 24 hours and 7 days at 4°C and isolations were done according to the protocol.

[a] HE-Hektoen Agar: MAC-MacConkey's Agar: XLD-xylose-lysine-deoxycholate agar

[b] From enrichment broth Selenite/GN broth

[c] = No salmonellae recoverable

[d] = Recoverable salmonellae

TABLE 15-5

Media which yielded the recovery of *Salmonella sp*. from sludge and human specimens

Sample Source	Species	Primary[a]	Enrichment		
			GN	Selenite	Tetrathionate
Human --	*muenchen*	-	-	+	-
Human --	*java*	+	-	+	-
Med. 300[b] --	*St. Paul*	+	+	-	-
	newington	-	-	+	-
Med. 500 --	*infantis*	-	-	+	-
Zan. --	*hinshawii*	-	-	+	-
Cols. --	*derby*	-	-	+	-
Med. 300 --	*San diego*	-	-	+	-
Human --	*muenchen*	+	-	+	-
Med. 500 --	*oranienburg*	+	-	-	-
Cols. --	*untypable*	-	-	-	+
Cols. --	*agona*	-	-	+	-
Cols. --	*agona*	-	-	+	-
Cols. --	*infantis*	-	-	+	-
Cols. --	*untypable*	-	-	-	-
Cols. --	*agona*	-	-	+	-
Cols. --	*infantis*	+	-	+	-
Cols. --	*anatum*	-	-	+	-
Cols. --	*thompson*	-	-	+	-
Cols. --	*ohio*	-	-	+	-
Cols. --	*enteritidis*	-	-	+	-
Cols. --	*enteritidis*	-	-	+	-
Med. 300 --	*st. paul*	-	-	+	-
Med. 500 --	*st. paul*	-	+	-	-
Spfld. --	*infantis*	-	-	+	-

[a] Primary isolation is marked + if recovery was made on HEK, XLD or MAC plates. If recovery was seen following enrichment in GN, selenite or tetrathionate broths, the corresponding column is marked.

[b] Med. 300 = Medina 300 plant; Med. 500 = Medina 500 plant; Cols. = Columbus; Spfld = Springfield; Zan = Zanesville.

TABLE 15-6

Campylobacter Recovery - Seeded Sludge from Medina 300 Plant

Contact[a] Time	No. org. added	15,000 CFU/ml		15,000 CFU/ml				150 CFU/ml	
1 hr.	−[c]	+[d]	+	+	+	+	+	+	+
24 hr.	−	+	+	−	+	+	+	−	+
24 hr.[b]	−	+	+	−	+	+	+	−	+
7 days	−	+	+	−	+	+	+	−	+

[a] Campylobacter foetus ssp. jejuni was inoculated in sludge to the final concentration indicated and held for a specified time at 4°C before plating.

[b] Subcultured in Campy Thioglycollate broth

[c] No Campylobacter colonies recognized

[d] Campylobacter confirmed

TABLE 15-7

Campylobacter Recovery - Seeded Sludge from Medina 500 Plant

Contact[a] Time	No. org. added	75,000 CFU/ml		75,00 CFU/ml		750 CFU/ml	
1 hr.	-[b]	+[c]	+	+	+	+	+
24 hr.	-	+	+	+	+	+	+
7 days	-	+	+	+	+	-	+

[a] *Campylobacter foetus ssp. jejuni* was inoculated in sludge to the final concentration indicated and held for a specified time at 4°C before plating.

[b] No *Campylobacter* colonies recognized

[c] *Campylobacter* confirmed

TABLE 15-8

Campylobacter Recovery - Seeded Sludge from Columbus Plant

Contact[a] Time	No. org. added	ORIGINAL FINAL CONCENTRATION					
		10,000 CFU/ml		1,000 CFU/ml		100 CFU/ml	
1 hr.	-[b]	+[c]	+	+	+	-	-
24 hr.	N.A.[d]						
4 days	-	+	+	+	+	-	-
7 days	-	+	+	+	-	-	-

[a] *Campylobacter foetus ssp. jejuni* was inoculated in sludge to the final concentration indicated and held for a specified time at 4°C before plating.

[b] No *Campylobacter* colonies recognized

[c] *Campylobacter* confirmed

[d] N.A. Not available.

TABLE 15-9

Campylobacter Recovery - Seeded Sludge from Springfield Plant

		ORIGINAL FINAL CONCENTRATION					
Contact[a] Time	No. org. added	10,000 CFU/ml		1,000 CFU/ml		100 CFU/ml	
1 hr.	-[c]	+[d]	+	+	+	+	-
24 hr.	-	+	+	+	+	+	+
24 hr.[b]	-	+	-	-	+	-	-
7 days	-	-	-	-	-	-	-

[a] *Campylobacter foetus ssp. jejuni* was inoculated in sludge to the final concentration indicated and held for a specified time at 4°C before plating.

[b] subcultured in Campy Thioglycollate broth

[c] No *Campylobacter* colonies recognized

[d] *Campylobacter* confirmed

TABLE 15-10

Salmonellae Isolations from Sludge by quarter and year for the Medina 300, 500 and Springfield plant

Quarter		Serotype
	Medina 300	
1 -- 1979		*st. paul*
2 -- 1979		*newington*
3 -- 1979		*san diego*
4 -- 1979		*st. paul*
1 -- 1980		*enteritidis*
2 -- 1980		*typhimurium*
1 -- 1981		*untypable*
1 -- 1981		*tenessee*
2 -- 1981		*java*
2 -- 1981		*typhimurium*
	10 isolates from 64 sludges tested	
	Medina 500	
2 -- 1979		*infantis*
3 -- 1979		*oraniensburg*[a]
1 -- 1980		*st. paul*
2 -- 1980		*untypable*
1 -- 1981		*oraniensburg*[a]
	5 isolates from 64 sludges tested	
	Springfield	
1 -- 1980		*infantis*
2 -- 1980		*reading*
3 -- 1981		*infantis*
3 -- 1981		*agona*
	4 isolates from 58 sludge samples	

---

[a] There are differences in the antibiogram of the two strains of *S. oranienburg* isolated in 1979 and 1981. The 1981 strain showed marked resistance to tetracycline (>128) and to chloraphenicol (>16)

TABLE 15-11

Salmonellae isolations by quarter and year for the Columbus Plant

Quarter	Serotype
Columbus Jackson Pike	
2 -- 1979	*derby*
3 -- 1979	untypable
4 -- 1979	*agona*
4 -- 1979	*agona*
4 -- 1979	*infantis*
4 -- 1979	untypable
4 -- 1979	*agona*
1 -- 1980	*infantis*
1 -- 1980	*anatum*
1 -- 1980	*thompson*
1 -- 1980	*ohio*
1 -- 1980	*enteritidis*
2 -- 1980	*st. paul*
3 -- 1980	nontypable
3 -- 1980	*panama*
4 -- 1980	*typhimurium*
1 -- 1981	*montevideo*
1 -- 1981	*infantis*
1 -- 1981	*adelaide*
1 -- 1981	*ohio*
1 -- 1981	*thompson*
1 -- 1981	*montevideo*
Columbus Jackson Pike	
2 -- 1981	*manhattan*
2 -- 1981	*infantis*
2 -- 1981	*havana*
4 -- 1981	*binza*
4 -- 1981	*montevideo*
4 -- 1981	*reading*
1 -- 1982	*oranienburg*
1 -- 1982	*typhimurium*
1 -- 1982	*infantis*
31 isolates from 125 sludges tested	

TABLE 15-12

Summary of Salmonellae Isolations by Site

		Positive/tested	% positive
Medina 300		10/64	15.6
Medina 500		5/64	7.8
Columbus		31/125	25
Springfield		4/58	7
	Totals	50/311	16%

TABLE 15-13

Salmonellae Serotypes Isolated from All Sites

Serotype	Frequency	Percent
adelaide	1	2
agona	4	8
anatum	1	2
binza	1	2
derby	1	2
enteritidis	2	4
havana	1	2
infantis	8	16
java	1	2
manhattan	1	2
montevideo	3	6
newington	1	2
ohio	2	4
oranienburg	3	6
panama	1	2
reading	2	4
st. paul	4	8
san diego	1	2
tenessee	1	2
typhimurium	4	8
thompson	2	4
non-typable	5	10

TABLE 15-14

Salmonellae Isolation by Quarters

Positive/tested	Quarter 1	Quarter 2	Quarter 3	Quarter 4
	22/92	11/69	7/62	10/88

Salmonellae Isolation by Quarter and by Site

Site	Quarter 1	Quarter 2	Quarter 3	Quarter 4
Medina 300	5/19	3/15	1/13	1/17
Medina 500	2/18	2/14	1/13	0/18
Columbus	14/37	5/28	3/25	9/36
Springfield	1/18	1/12	2/11	0/17

TABLE 15-15

Salmonellae Isolations from Columbus Sludge by Quarters

	Quarter 1	2	3	4
# Positive per quarter / Total # Columbus Positives	14/31[a]	5/31	3/31	9/31

[a] Significantly different (p = <0.05) when compared by a 2 x 2 chi square vs. all other quarters together.

TABLE 15-16

Distribution of MICs for Salmonellae Isolated from Sludge

	MIC (% of isolates)[a]											
Antibiotic	<=0.5	0.5	<=1	1	<=2	2	4	8	16	>32	>64	>128
Ampicillin	2			40		46	5	2		2		
Carbenicillin[b]					10		80					10
Ticarcillin					28		58	9				5
Cephalothin			2		16	44	30	7				
Cefamandole	25	63		7		5						
Cefoxitin			9		16	28	42	5				
Gentamicin	67			26		2	5					
Tobramycin	37			44		12	7					
Amikacin			35			42	14	9				
Tetracycline			12			70	9					7
Chloramphenicol							23	19	5	2		

n = 43 isolates tested for antibiotic sensitivity

[a] rounded out to nearest %

[b] n = 21

TABLE 15-17

Multiple Resistance to Antibiotics in Salmonallae Isolated from Sludge

		MIC ug/ml										
SOURCE	SEROTYPE	AMPIC	CARBE	TICAR	CEFAL	CEFAM	CEFOX	GENTA	TOBRA	AMIKA	TETRA	CHLOR
Med. 500	*oranienburg*	2	4	<=2	<=2	<=.5	4	4	4	8	>128	>16
Cols.	*infantis*	>32	>128	>128	8	2	8	<=.5	1	2	>128	8
Cols.	*typhimurium*	>32	ND	>128	8	2	2	1	2	4	>128	4
Cols.	*montevideo*	8	ND	8	8	1	4	<=.5	1	<=1	4	16
Spfld.	*agona*	2	ND	8	4	.5	4	<=.5	1	2	4	16

ND = not done

TABLE 15-18

Isolation of Salmonellae from Human Stool Specimens 1978-82

Subject #	Source of Sludge	Date	Species
100901	Medina	12/5/78	muenchen
150602	Medina	12/11/78	java
400406	Columbus	7/24/79	oranienburg
401704	Columbus	11/18/81	enteritidis
401704	Columbus	12/3/81	enteritidis

TABLE 15-19

Subjects with agglutinating antibodies to Salmonellae serotypes

Salmonellae Serologic Group	Medina	Franklin	COUNTY Pickaway	Clark	Total
B	8/50	0/39	11/111	1/62	20/262
C	25/50	8/39	14/111	18/62	65/262
D	8/50	0/39	18/111	7/62	33/262
E	8/50	1/39	9/111	5/62	23/262

Number of subjects with antibodies to salmonellae serotypes over number of subjects in a given county.

TABLE 15-20

Conversions[a] and Rises[b]

Salmonellae Serologic Group	Medina	Franklin	Pickaway	Clark
B	0	0	1 rise 2 conversions	0
C	2 conversions	0	0	0
D	0	0	1 rise	0
E	0	0	2 conversions	0

[a] Conversions - Serological conversion from negative to positive in paired samples

[b] Rise - Increase by 2 grades of reactivity in paired samples

TABLE 15-21

Examples of Family Patterns of Antibodies to Salmonellae O Antigens

Subject #	Group B	C	D	E
100401	+ [a]	+	+	- [b]
100402	-	-	+	-
401701	-	-	-	-
401702	-	-	-	-
401703	-	-	-	-
401704 [c]	rise	-	-	rise
401705	-	-	-	-
100801	-	+	-	-
100802	-	+	+	+
100804	-	conversion	+	+
100805	-	+	+	+
100806	+	+	-	+

[a] + agglutination seen (1+ to 4+) at a serum dilution of 1:20.

[b] no agglutination seen

[c] rises followed infection with Salmonella enteritidis. (2 isolations from stools). No serial dilutions were done so the negative result with group D antigen might be the result of prozone effect.

TABLE 15-22

Stool Samples Examined[a] for Ova and Parasites According to Year of Study, Location and Study Status

Year	No. of Stools Examined Columbus Sludge	 Columbus Controls	 Medina Sludge	 Medina Controls	 Springfield Sludge	 Springfield Controls	Totals
1978	0	0	20	13	0	0	33
1979	0	0	0	0	0	0	0
1980	89	64	22	21	23	17	236
1981	63	43	3	5	14	10	138
Totals	152	107	45	39	37	27	407

[a] 26% of 1,556 samples were examined

TABLE 15-23

Ova and Parasites Found in Sludge According to Year and Source

Location	No. of Sludge Samples Tested 1980	1981	1982	Totals	No. Positive for Mites	Mite ova	Other ova
Columbus	11	14	2	27	2 (7.4%)	6 (22.2%)	5 toxocara (18.5%)
Springfield	4	4	0	8	0	0	0
Medina 300	14	11	0	25	8 (32%)	3 (12%)	1 Unidentified (4%)
Medina 500	15	7	0	22	12 (54.5%)	2 (9.1%)	1 Unidentified (4.5%)

TABLE 15-24

Viruses and Cell Cultures Used In Serum Microneutralization Tests

Virus	Cell Line
coxsackievirus A3,7,9,11,15	RD[a]
echovirus 7,21,24	RD
coxsackievirus B1-6	BGM[b]
echovirus 3,6,9,11,12,19,20,25,26	BGM

[a] Human rhabdomyosarcoma

[b] Buffalo green monkey kidney

TABLE 15-25

Identities of Enteric Viruses and Frequency of Isolation from Sludge Samples[a]

Medina 300 Treatment Plant

Virus	Year 1	Year 2	Year 3[b]	Totals
Echo 5	0	0	3	3
Echo 6[c]	0	1	0	1
Echo 7[c]	19	9	0	28
Echo 11[c]	1	1	5	7
Echo 19	1	0	0	1
Echo 24[c]	0	1	0	1
Echo 27	1	0	0	1
Echo 30	1	0	0	1
Coxsackie B2[c]	1	2	1	4
Coxsackie B3[c]	1	9	5	15
Coxsackie B4[c]	0	1	2	3
Reovirus	3	0	0	3
Poliovirus 1	0	2	0	2
Poliovirus 2	3	7	7	17
Poliovirus 3	2	2	0	4
Totals	33	35	23	91

[a] n = 63, 58 (92%) of the samples yielded one or more viruses, mean of 1.57 viruses per positive sample

[b] Year 3 was only 8 months long.

[c] These viruses were included in serum neutralization tests with human sera.

TABLE 15-26

Multiple Viral Isolates from 30 M300 Sludge Samples

Viral Combinations Isolated[a]	Frequency of Occurrence
CB3, CB4	1
CB2, E7	3
CB3, E7	6
CB3, E11	2
CB3, P1	1
CB4, E11	1
E5, P2	2
E7, E11	1
E7, P2	4
E7, reovirus	1
E19, E27	1
P1, P2	1
P2, P3	2
P3, reovirus	1
E7, P2, reovirus	1
CB2, CB3, E11	1
CB3, E11, P2	1

---

[a] CB - coxsackievirus B
E - echovirus
P - poliovirus

TABLE 15-27

Identities of Enteric Viruses and Frequency of Isolation From Sludge Samples[a]

Medina 500 Treatment Plant

Virus	Year 1	Year 2	Year 3	Totals
Echo 5	1	0	0	1
Echo 6[c]	0	1	0	1
Echo 7[c]	11	9	2	22
Echo 11[c]	0	2	1	3
Echo 13	1	0	0	1
Echo 24[c]	0	1	0	1
Echo 25[c]	1	0	0	1
Echo 27	0	1	0	1
Echo 30	0	0	1	1
Coxsackie B3[c]	0	4	6	10
Coxsackie B4[c]	0	1	2	3
Reovirus	2	-	-	2
Poliovirus 1	0	2	0	2
Poliovirus 2	2	3	1	6
Poliovirus 3	0	1	3	4
Totals	18	25	16	59

[a] n = 63 tested, 43 positive for 1 or more viruses (68%), a yield of 1.37 viruses per positive sample.

[b] Year 3 was only 6 months long.

[c] These viruses included in serum neutralization tests with human sera.

TABLE 15-28

Multiple Viral Isolates from 15 M500 Sludge Samples

Viral Combinations Isolated[a]	Frequency of Occurrence
CB3, CB4	1
CB3, E7	4
CB3, E11	1
CB4, E7	1
E5, E7	1
E7, P3	1
E25, reovirus	1
P1, P2	2
P2, P3	1
P2, reovirus	1
CB3, CB4, P3	1

[a] CB - coxsackievirus B
E - echovirus
P - poliovirus

TABLE 15-29

Identities of Enteric Viruses and Frequency of Isolation from Sludge Samples[a]

Columbus Treatment Plant

Virus[b]	Year 1	Year 2	Year 3	Totals
Echo 3[c]	0	0	4	4
Echo 5	0	3	0	3
Echo 6[c]	1	2	0	3
Echo 7[c]	8	3	2	13
Echo 11[c]	0	1	0	1
Echo 15	0	1	0	1
Echo 17	0	1	0	1
Echo 19[c]	0	0	1	1
Echo 20[c]	0	1	4	5
Echo 21[c]	1	2	1	4
Echo 22	0	0	1	1
Echo 24[c]	0	12	1	13
Echo 25[c]	0	0	1	1
Echo 27	0	2	0	2
Echo 30	0	1	3	4
Coxsackie A9[c]	0	1	2	3
Coxsackie A16	1	0	0	1
Coxsackie B2[c]	2	0	2	4
Coxsackie B3[c]	0	14	1	15
Coxsackie B4[c]	0	1	1	2
Coxsackie B5[c]	2	1	1	4
Polio 1	1	1	1	3
Polio 2	5	2	8	15
Polio 3	2	0	3	5
Totals	23	49	37	109

[a] n = 123 tested, 81 (66%) positive for 1 or more viruses, mean of 1.35 viruses per positive sample.

[b] No reoviruses are listed probably because primary monkey kidney cells were not used to test these samples.

[c] These viruses included in serum neutralization tests with human sera.

TABLE 15-30

Multiple Viral Isolates from 28 Columbus Sludge Samples

Viral Combinations Isolated	Frequency of Occurrence
CB2, E7	2
CB2, E24	1
CB3, E5	2
CB3, E7	1
CB3, E24	8
CB4, E3	1
CB5, E6	1
CB5, E20	1
CB5, E24	1
CB5, P3	1
CA9, P2	1
CA16, P1	1
E20, E24	1
E20, E27	1
E25, P1	1
E25, P2	1
E30, P1	1
P2, P3	2

CB - coxsackievirus B
CA - coxsackievirus A
E - echovirus
P - poliovirus

TABLE 15-31

Identities of Enteric Viruses and Frequency of Isolation from Sludge Samples[a]

Springfield Plant

Virus	Year 1	Year 2	Totals
Echo 3[c]	0	2	2
Echo 6[c]	2	0	2
Echo 7[c]	2	0	2
Echo 13	3	0	3
Echo 21[c]	0	1	1
Echo 24[c]	2	1	3
Echo 25[c]	0	1	1
Echo 27	1	0	1
Echo 30	0	1	1
Coxsackie A16	0	1	1
Coxsackie B2[c]	1	0	1
Coxsackie B3[c]	7	0	7
Coxsackie B4[c]	0	1	1
Coxsackie B5[c]	0	3	3
Polio 1	1	0	1
Polio 2	3	4	7
Polio 3	0	1	1
Totals	22	16	38

[a] n = 58 tested, 29 (50%) positive for 1 or more viruses, mean of 1.31 viruses per positive sample.

[b] No reoviruses were isolated listed probably because primary monkey kidney cells were not used to test these samples.

[c] These viruses included in serum neutralization with human sera.

TABLE 15-32

Multiple Viral Isolates from 8 Springfield Sludge Samples

Viral Combinations Isolated[a]	Frequency of Occurrence
CB3, E13	1
CB3, E24	2
CB3, P2	1
E13, E27	1
E13, P2	1
E24, P2	1
CB2, CB3, P1	1

[a] CB - coxsackievirus B
E - echovirus
P - poliovirus

TABLE 15-33

Frequency of Single, Double and Triple Viral Isolations from All Sludge Samples (n = 307)

Number of Samples Yielding			No. of Samples Positive	Total No. of viruses isolated
1 virus	2 viruses	3 viruses		
130 (42%)	76 (25%)	5 (2%)	211	297

TABLE 15-34

Summary of Viral Isolations[a] from all Sludge Samples (n = 307)[b] According to Serotype and Location

Virus	Medina 300 (n=63)	Medina 500 (n=63)	Columbus (n=123)	Springfield (n=58)	Totals
Echo 3	0	0	4	2	6
Echo 5	3	1	3	0	7
Echo 6	1	1	3	2	7
Echo 7	28	22	13	2	65
Echo 11	7	3	1	0	11
Echo 13	0	1	0	3	4
Echo 15	0	0	1	0	1
Echo 17	0	0	1	0	1
Echo 19	1	0	1	0	2
Echo 20	0	0	5	0	5
Echo 21	0	0	4	1	5
Echo 22	0	0	1	0	1
Echo 24	1	1	13	3	18
Echo 25	0	1	1	1	3
Echo 27	1	1	2	1	5
Echo 30	1	1	4	1	7
Coxsackie A9	0	0	3	0	3
Coxsackie A16	0	0	1	1	2
Coxsackie B2	4	0	4	1	9
Coxsackie B3	15	10	15	7	47
Coxsackie B4	3	3	2	1	9
Coxsackie B5	0	0	4	3	7
Reovirus	(3)	(2)	-	-	(5)[c]
Polio 1	2	2	3	1	8
Polio 2	17	6	15	7	45
Polio 3	4	4	5	1	14
Totals	91	59	109	38	297

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[a] This number includes multiple viral isolates

[b] 211 samples were positive (69%) for one or more viruses

[c] This number is low probably because primary monkey kidney cultures were not used throughout.

TABLE 15-35

Frequency of Viral Isolations from all Sludge Samples Tested (n = 307) According to Cell Culture[a]

Virus	RD	BGM	HeLa	CMK[b]	Total No. Isolated[c]
Echo 3	6	0	0	-	6
Echo 5	5	2	0	-	7
Echo 6	7	0	0	-	7
Echo 7	64	1	0	-	65
Echo 11	10	1	0	-	11
Echo 13	4	0	0	-	4
Echo 15	1	0	0	-	1
Echo 17	1	0	0	-	1
Echo 19	1	1	0	-	2
Echo 20	5	0	0	-	5
Echo 21	2	2	1	-	5
Echo 22	1	0	0	-	1
Echo 24	16	0	2	-	18
Echo 25	2	0	1	-	3
Echo 27	3	0	2	-	5
Echo 30	7	0	0	-	7
Coxsackie A9	3	0	0	-	3
Coxsackie A16	2	0	0	-	2
Coxsackie B2	0	9	0	-	9
Coxsackie B3	1	8	39	-	48
Coxsackie B4	5	2	2	-	9
Coxsackie B5	0	0	7	-	7
Reovirus	0	0	0	(5)	5
Polio 1	2	1	5	-	8
Polio 2	25	11	17	-	53
Polio 3	6	7	1	(1)	15
Totals	179	45	77	(6)	307

[a] RD = stable cell line derived from a human rhabdomyosarcoma.
BGM = stable cell line derived from green monkey kidneys.
CMK = primary cynomolgous monkey kidney

[b] these cells were used to test only 90/307 sludge samples.

[c] Numbers include results from samples (n = 10) in which the same virus was isolated from the same sample in more than one cell culture system.

TABLE 15-36

Summary of All Viruses Isolated from Sludge According to Cell Culture System(s)

Cell Culture(s)[a]	Viruses Isolated[b]
RD only	A9, A16, E3, E6, E13, E15, E17, E20, E22,E30
BGM only	CB2
PCMK[c] only	Reovirus
HeLa only	CB5
RD and BGM	E5, E7, E11, E19
RD and HeLa	E24, E24, E27
RD, BGM and HeLa	CB3, CB4, E21, P1, P2, P3

[a] RD - rhabdomyosarcoma,
BGM - Buffalo green monkey kidney
PCMK - primary cynomolgous monkey kidney

[b] CA - coxsackievirus A
CB - coxsackievirus B
E - echovirus
P - poliovirus

[c] Only 90 of 307 sludge samples inoculated onto these cells.

TABLE 15-37

Seasonal Effects on Viral Isolation Rates from Sludge Medina 500 Plant (no. positive/no. tested)

	July	Aug.	Sept.	Oct.	Totals[a]
Year 1	3/3	2/2	2/2	2/2	16/17 (94%)
Year 2	1/2	2/2	2/2	2/2	
	Jan.	Feb.	March	April	Totals
Year 1	1/3	1/1	0/3	0/2	7/16 (44%)
Year 2	0/1	2/2	2/2	1/2	

[a] $X^2 = 9.90$, $P = <0.005$, difference is significant

TABLE 15-38

Seasonal Effects on Viral Isolation Rates from Sludge
Columbus Plant (no. positive/no. tested)

	July	Aug.	Sept.	Oct.	Totals[a]
Year 1	4/4	3/3	4/4	4/4	25/32 (78%)
Year 2	1/4	2/5	3/4	4/4	

	Jan.	Feb.	March	April	Totals
Year 1	1/2	2/4	3/6	4/4	28/42 (66.7%)
Year 2	3/4	4/4	2/5	1/3	
Year 3	4/5	3/4	1/1		

[a] $X^2 = 1.17$, $P = < 0.3$, difference not significant

TABLE 15-39

Seasonal Effects on Viral Isolation Rates from Sludge Springfield Plant (no. positive/no. tested)

	July	Aug.	Sept.	Oct.	Totals[a]
Year 1	1/1	2/2	2/2	2/2	13/15 (87%)
Year 2	2/2	2/2	0/2	2/2	

	Jan.	Feb.	March	April	Totals
Year 1	0/2	0/2	1/2	0/2	5/20 (25%)
Year 2	0/2	1/2	2/2	0/2	
Year 3	0/2	0/2	1/2		

[a] $\chi^2 = 13.05$, $P = <0.0005$, difference is significant

TABLE 15-40

Frequency and Identifications of Viral Isolates from Stool Samples (n = 1,743) According to Study Status and Source of Sludge

Stools from Sludge Recipients		Stools from Controls	
Study No.	Virus	Study No.	Virus
	Medina		
100603	Coxsackievirus B5	150501	Echovirus 7
100603	Echovirus 27	150502	Echovirus 27
101001	Echovirus 27	150504	Unidentified
		150201	Echovirus 7
	Columbus		
300401	Echovirus 7	350801	Echovirus 26
300503	Echovirus 27	350802	Echovirus 26
300504	Echovirus 7	451303	Coxsackievirus A16
300601	Echovirus 7	451503	Coxsackievirus A9
300701	Echovirus 7	451808	Echovirus 22
300706	Echovirus 7		
300707	Unidentified		
300903	Echovirus 7		
400305	Echovirus 25		
400401	Echovirus 7		
400403	Echovirus 7		
401705	Coxsackievirus A9		
401802	Echovirus 27		
	Springfield		
0		550701	Echovirus 26

TABLE 15-41

Observed Frequency Table[a] of Any Virus Isolated[b] from a Subject at Anytime During Study

	Positive	Negative	Totals
Positive	5	6	11
Negative	3	32	35
Totals	8	38	46

[a] Each observation in cells is for paired farms, numbers in margins are for individual sludge and control farms. $X^2 = 1$, $P = <0.32$.

[b] Repeat isolations in a family were not considered. A positive farm is one from which 1 or more viruses was isolated at anytime during the course of the study.

TABLE 15-42

Comparative Analysis[a] of the Number of Susceptible Subjects in Sludge and Control Groups

Virus[b]	Sludge Mean	Control Mean	p value
CB1	.66	.83	.02
CB2	.57	.48	.19
CB3	.63	.72	.26
CB4	.38	.38	.81
CB5	.71	.77	.39
CB6	.99	.99	1.00
CA3	.28	.26	.60
CA7	.49	.53	.58
CA9	.74	.78	.64
CA11	1.00	1.00	-
CA15	.97	.96	1.00
E3	.64	.65	.84
E6	.72	.60	.07
E7	.80	.80	.99
E9	0.00	0.00	-
E11	.52	.53	.83
E12	.66	.69	.60
E19	.65	.59	.54
E20	.65	.63	.62
E21	.63	.71	.20
E25	.30	.39	.23
E26	.57	.51	.41
E24	.84	.85	.95

[a] Wilcoxen Sign-Rank test
[b] C = coxsackievirus, E= echovirus

TABLE 15-43

Serum Neutralizing Antibody Rises Detected in All Subjects During the Course of the Study

Person[a]	Dates sera were collected	Virus[b]	Antibody titers[c] of paired sera
	Medina County		
100401	8/9/78 - 12/5/78	EC9	0 - 80
100401	8/9/78 - 12/5/78	EC25	<2.5 - 10
100401	3/13/79 - 8/7/79	CB3	0 - 160
100401	3/13/79 - 8/7/79	EC24	20 - 320
100401	3/13/79 - 8/7/79	CB1	5 - 80
100402	8/8/78 - 12/5/78	CB5	10 - 80
100402	8/8/78 - 12/5/78	CB6	0 - 20
100402	8/8/78 - 12/5/78	CA9	<2.5 - 10
100402	8/8/78 - 12/5/78	EC3	<2.5 - 10
100402	8/8/78 - 12/5/78	EC11	10 - 40
100402	8/8/78 - 12/5/78	EC12	10 - 160
100402	12/5/78 - 3/13/79	EC7	10 - 40
100402	12/5/78 - 3/13/79	CB2	10 - 40
100402	3/13/79 - 8/7/79	EC19	0 - 40
100201	8/2/79 - 12/7/79	EC3	40 - 160
100201	8/2/79 - 12/7/79	EC20	40 - 160
100202	4/27/78 - 8/8/78	EC9	20 - 80
100202	8/8/78 - 12/5/78	CA3	80 - 320
100202	4/1/80 - 8/5/80	EC21	<2.5 - 10
100202	8/5/80 - 12/4/80	CB3	0 - 40
100301	4/1/80 - 9/18/80	EC25	<2.5 - 10
100601	7/31/79 - 12/7/79	EC25	40 - 160
100602	3/13/80 - 8/2/80	EC25	40 - 160
100603	4/12/78 - 8/8/78	CB5	0 - 20
100603	7/31/79 - 12/7/79	EC25	0 - 40
101001	8/9/78 - 12/5/78	EC25	0 - 320
101002	4/7/78 - 8/9/78	EC20	80 - 320
101002	12/5/78 - 3/13/79	EC25	10 - 40
101002	8/7/79 - 12/7/79	EC11	40 - 320
150301	8/8/78 - 12/5/78	EC11	0 - 160
150302	4/11/78 - 8/8/78	CB5	40 - 160
150303	12/7/79 - 3/12/79	CA3	0 - 20
150304	8/2/79 - 12/7/79	CB3	0 - 160

TABLE 15-43, Con't.

Person	Dates sera were collected	Virus	Antibody titers of paired sera
150501	7/31/79 - 12/7/79	EC7	10 - 40
150501	8/8/79 - 12/5/78	EC25	0 - 20
150502	4/13/78 - 8/9/78	EC3	<2.5 - 10
150502	4/13/78 - 8/9/78	EC6	<2.5 - 10
150502	4/13/78 - 8/9/78	EC20	<2.5 - 10
150502	8/9/78 - 12/5/79	EC21	20 - 320
150502	8/9/78 - 12/5/79	EC25	0 - 20
150502	12/5/78 - 3/19/79	EC25	20 - 80
150502	12/7/79 - 4/2/80	EC7	<2.5 - 10
150601	12/7/79 - 4/15/80	EC9	10 - 80
150701	4/7/78 - 8/8/78	CA7	20 - 80
150801	8/8/78 - 12/5/78	EC25	0 - 80
150801	8/8/78 - 12/5/8	EC11	0 - 80
150801	7/31/70 - 12/7/79	EC9	40 - 160
150802	12/5/78 - 3/16/79	CB5	0 - 40
	Franklin Co.		
300201	6/3/80 - 10/29/80	EC24	0 - 40
300201	6/13/79 - 10/22/79	EB3	0 - 80
300202	6/3/80 - 10/29/80	EC24	0 - 80
300203	6/13/79 - 10/22/79	CB3	0 - 20
300203	6/3/80 - 10/29/80	EC24	0 - 320
300204	6/13/79 - 10/22/79	CB5	20 - 80
300204	6/13/79 - 10/22/79	CB3	0 - 160
300204	6/3/80 - 10/29/80	EC24	0 - 320
300303	6/3/80 - 10/30/80	CB3	<2.5 - 10
300304	6/3/80 - 10/30/80	CB3	0 - 320
300401	11/6/79 - 3/17/80	CA7	<2.5 - 10
300501	6/23/81 - 12/2/81	CA3	10 - 40
300602	11/4/80 - 3/13/81	CB4	5 - 20
300902	7/21/81 - 11/19/81	EC3	<2.5 - 10
300902	7/21/81 - 11/19/81	EC12	<2.5 - 10
300902	7/21/81 - 11/19/81	EC26	<2.5 - 10
350101	8/18/81 - 12/17/81	EC25	0 - 40

TABLE 15-43, Con't.

Person	Dates sera were collected	Virus	Antibody titers of paired sera
350201	8/1/80 - 12/15/80	CB2	10 - 40
350801	6/3/81 - 9/28/81	CB4	0 - 160
350801	6/3/81 - 9/28/81	CB5	10 - 320
350802	6/3/81 - 9/28/81	CA7	0 - 10
351001	7/27/81 - 11/23/81	CB2	10 - 40
351001	7/27/81 - 11/23/81	EC21	5 - 20
351002	7/27/81 - 11/23/81	EC11	<2.5 - 10
	Pickaway Co. - Columbus Sludge		
400201	11/14/79 - 4/7/80	EC11	0 - 40
400201	11/14/79 - 4/7/80	CB1	0 - 40
400201	11/14/79 - 4/7/80	CB3	0 - 40
400201	11/14/79 - 4/7/80	EC25	0 - 80
400202	11/10/80 - 4/15/81	CA3	0 - 40
400202	11/10/80 - 4/15/81	CB4	0 - 20
400202	4/15/81 - 8/11/81	CB2	5 - 20
400404	11/19/79 - 3/20/80	CB5	0 - 80
401205	2/12/80 - 6/16/80	EC25	5 - 20
401403	6/23/80 - 10/14/80	CA3	<2.5 - 10
401701	3/27/79 - 7/16/79	CA7	<2.5 - 10
401804	10/31/79 - 2/25/80	EC21	5 - 20
401804	2/25/80 - 6/10/80	CA7	10 - 160
450401	7/10/79 - 11/1/79	EC19	0 - 20
450401	7/10/79 - 11/1/79	EC20	0 - 20
450502	3/16/81 - 7/20/81	CA3	40 - 320
451202	5/28/80 - 9/22/80	EC21	5 - 20
451206	9/22/80 - 1/12/81	EC25	40 - 160
451302	4/14/80 - 8/13/80	CB3	<2.5 - 10
451303	4/14/80 - 12/15/80	CB3	<2.5 - 10
451303	4/14/80 - 12/15/80	CB4	10 - 40
451303	4/14/80 - 12/15/80	CB1	<2.5 - 10
451304	4/14/80 - 8/13/80	CB3	0 - 40

TABLE 15-43, Con't.

Person	Dates sera were collected	Virus	Antibody titers of paired sera
451601	7/14/81 - 11/18/81	CB4	0 - 20
451701	66/24/81 - 10/14/81	EC20	0 - 40
451503	7/11/79 - 11/29/79	CB1	0 - 40
451503	7/11/79 - 11/29/79	CB5	0 - 20
451503	7/11/79 - 11/29/79	EC6	0 - 80
451503	7/11/79 - 11/29/79	EC12	<2.5 - 10
	Clark Co. - Springfield Sludge		
500305	7/19/81 - 12/8/81	EC6	0 - 80
500601	12/29/81 - 4/19/82	EC12	<2.5 - 10
500701	7/22/81 - 11/20/81	CB5	0 - 20
500701	7/22/81 - 11/20/81	EC11	<2.5 - 5
500701	11/20/81 - 4/13/82	CB2	<2.5 - 10
500701	11/20/81 - 4/13/82	CA3	5 - 160
500702	7/22/81 - 11/20/81	CB2	0 - 20
500702	7/22/81 - 11/20/81	CA3	10 - 160
500702	11/20/81 - 4/13/82	EC9	<2.5 - 10
500801	3/2/81 - 6/24/81	CB4	40 - 320
500803	2/27/81 - 6/24/81	CA3	0 - 320
550101	11/18/81 - 3/17/82	CA3	<2.5 - 10
550204	8/13/80 - 12/18/80	CA7	0 - 40
550205	8/13/80 - 12/18/80	CB1	0 - 40
550205	8/13/80 - 12/18/80	EC21	0 - 40
550602	11/19/80 - 3/17/81	EC26	80 - 320
550701	7/16/80 - 11/6/80	CA7	5 - 80
550702	7/16/80 - 11/6/80	CA7	<2.5 - 10
551204	8/25/81 - 12/15/81	CB1	0 - 20
551204	8/25/81 - 12/15/81	CB5	5 - 40

Con't.

TABLE 15-43, Con't.

[a] The first numeral indicates the study area. The second numeral indicates a sludge (0) or control (5) farm. The fourth numeral indicates the farm number.

[b] C = coxsackievirus, EC = echovirus

[c] 0 = less than 1:10

TABLE 15-44

Frequency Distribution of 124 Neutralizing Antibody Rises[a] Among 67 Subjects

No. of rises	No. of individuals
1	46
2	10
3	8
4	2
5	2
6	-
7	1
8	-
9	1

[a] 69 rises occurred in 34 sludge subjects.
55 rises occurred in 33 control subjects.

TABLE 15-45

Distribution of 124 Neutralizing Antibody Rises in 67 Subjects[a] According to Virus

Virus[a]	No. of Rises
CB1	6
2	6
3	12
4	6
5	10
6	1
CA3	10
7	10
9	1
11	2
15	0
E3	4
6	3
7	3
9	5
11	6
12	4
19	2
20	5
21	6
24	5
25	15
26	2

[a] CB = coxsackievirus B, CA = coxsackievirus A, E = echovirus

TABLE 15-46

Matched Pair[a] Logistic Regression Analysis of Fourfold Increases in Antibody Titer to Any of 23 Viral Antigens Within 6 Months of First Sludge Application

Variable	Multiple Logistic Model			Simple Logistic Models		
	Logistic Coeffocient	Standard Error	P Value	Logistic Coefficient	Standard Error	P Value
Presence of Sludge	.093	.488	.90	.073	.415	.86
Number of Weeks Between Blood Samples	.123	.112	.30	.173	.216	.31
Number of Persons on Farm[b]	.080	.165	.80	.020	.131	.86

[a] n = 46 pairs

[b] Only one family selected from multiple family farms for this analysis

TABLE 15-47

Matched Pair[a] Logistic Regression Analysis of Fourfold Increases in Antibody Titer to Any of 23 Viral Antigens Within 6 Months of Second Sludge Application

Variable	Multiple Logistic Model			Simple Logistic Models		
	Logistic Coefficient	Standard Error	P Value	Logistic Coefficient	Standard Error	P Value
Presence of Sludge	.077	.391	.88	.057	.313	.89
Number of Weeks between Blood Samples	.169	.231	.35	.151	.203	.37
Number of Persons on Farm[b]	-.0001	.065	.97	.008	.082	.98

[a] n = 29 pairs

[b] Only one family selected from multiple family farms for this analysis

TABLE 15-48

Matched Pair[a] Logistic Regression Analysis of Fourfold Increases in Antibody Titer to Any of 23 Viral Antigens at Any Time Between First Sludge Application and End of Project

Variable	Multiple Logistic Model			Simple Logistic Models		
	Logistic Coefficient	Standard Error	P Value	Logistic Coefficient	Standard Error	P Value
Presence of Sludge	.083	.344	.80	.046	.305	.87
Number of Blood Samples	.257	.220	.24	.194	.195	.32
Number of Persons on Farm[b]	.077	.157	.62	.017	.129	.89

[a] n = 46 pairs

[b] Only one family selected from multiple family farms for this analysis

TABLE 15-49

Antibody Rises in Matched Farm Pairs Following the First Application of Sludge

		Control Farm		
		Rise	No Rise	
Sludge Farm	Rise	15 Pairs	7 Pairs	22 Pairs
	No Rise	6 Pairs	18 Pairs	24 Pairs
		21 Pairs	25 Pairs	N = 46 Pairs

TABLE 15-50

Percent of 262 Individuals With Neutralizing Antibody to 23 Enteroviruses by Age

Virus	n=20 5-12	n=27 13-21	n=80 22-40	n=82 41-49	n=53 60+	n=262 All Ages
B1	20	26	21	27	21	23
2	40	33	50	40	47	42
3	15	15	30	27	38	25
4	30	44	60	60	62	51
5	25	26	28	20	25	25
6	0	0	3	0	0	0.6
Mean %	22	24	32	29	32	28
CA3	30	37	64	78	81	58
7	20	44	28	48	64	41
9	5	15	28	28	17	19
11	0	0	0	0	0	0
15	0	0	3	7	0	2
Mean %	11	19	25	32	32	24
E3	35	19	29	32	34	30
6	30	15	30	32	37	29
7	20	7	11	22	38	20
9	100	100	99	96	96	98
11	55	22	43	43	60	45
12	20	7	29	31	36	25
19	25	15	25	35	60	32
20	45	22	34	34	32	33
21	20	22	16	31	47	27
24	5	4	7	12	25	11
25	30	44	60	67	77	56
26	20	41	38	42	57	40
Mean %	34	27	35	40	50	37
TOTAL MEAN %	26	24	32	35	42	32

TABLE 15-51

Spread of Virus[a] in Households with Susceptible Individuals

Household #	Subject#	Antibody rises and titers						
		CB1	CA7	E21				
1	550201	<5	80	<5				
	550202	40	<5	40				
	550204	<5	<10-40	<5				
	550205	<10-40	<5	<10-40				
		CB1	CB5					
2	551202	<5	<5					
	551203	<5	<5					
	551204	<10-20	5-40					
		CB1	CB3	CB4				
3	451301	5	<5	<5				
	451302	40	<2.5-10	160				
	451303	<2.5-10	<2.5-10	10-80				
	451304	10	<10-40	80				
		CB3	CB5	E24				
4	300201	<10-80	160	<10-40				
	300202	80	80	<10-80				
	300203	<5-20	160	<10-320				
	300204	<10-160	20-80	<10-320				
		CB3						
5	300301	20						
	300302	<5						
	300303	<2.5-10						
	300304	<10-320						
		CB1	CB2	CB3	CB5	CB6	CA9	E3
6	100401	5-80	<5	<5-160	20	<5	<5	20
	100402	20	10-40	<5	10-80	<10-20	<5-10	<2.5-10
		E7	E9	E11	E12	E19	E24	
	100401	<5	<5-80	80	5	<5	20-320	
	100402	<10-40	5					

Con't.

TABLE 15-51, con't.

House-hold #	Subject #	Antibody rises and titers				
		CB3	CB5	CA3	E11	
7	150301	<5	<5	40	<5-160	
	150302	10	40-160	160	20	
	150303	<5	<5	<5-20	<5	
	150304	<10-160	<5	<5	<5	
	150305	<5	<5	<5	<5	
		E25	CB5			
8	100601	40-160	<5			
	100602	40	<5			
	100603	<5-40	<10-40			
		CB4	B5	CA7		
9	350801	<10-160	10-320	<5		
	350802	80	<5	<5-10		
		CA3				
10	401402	<5				
	401403	<2.5-10				
	401404	<5				
		CB2	CB5	CA3	E9	E11
11	500701	<2.5-10	<10-20	5-160	10	<2.5-10
	500702	< 10-20	40	10-160	<2.5-10	
		CA3				
12	400401	80				
	400402	<5				
	400403	<5-10				
	400404	<5				
		E19	E20			
13	450401	<10-20	<10-20			
	450402	< 5	< 5			
		CA7				
14	401701	<5-10				
	401702	<5				

Con't.

TABLE 15-51, Con't.

House-hold #	Subject #	Antibody rises and titers					
		E3	E6	E7	E20	E21	E25
15	150501	<5	<5	10-40	20	20	<10-20
	150502	<5-10	<5-10	<5-10	<5-10	20-320	<10-80
		E11	E20	E25			
16	101001	40	20	<10-320			
	101002	40-320	80-320	10- 40			
		E3	E12	E26			
17	300901	<5	80	<5			
	300902	5-10	<5-10	<5-10			
	300903	<5	<5	80			
		CB4	CB5	CA7			
18	350801	<10-160	10-320	<5			
	350802	80	<5	<5-10			
		CB5					
19	400401	<5					
	400402	20					
	400403	<5					
	400404	<10-80					
	400405	160					
		E21					
20	401801	5					
	401802	<5					
	401803	20					
	401804	5-20					
		E21	E25				
21	451201	<5	10				
	451202	5-20	10				
	451203	<5	<5				
	451204	<5	40				
	451205	<5	20				
	451206	<5	40-160				

Con't.

TABLE 15-51 cont'd

Household #	Subject #	Antibody rises and titers	
		E12	
22	451502	<5	
	451503	<5-10	
		CB4	CA3
23	500801	40-320	10
	500802	<5	<5
	500803	<5	<10-320
		CA7	
24	550701	5-80	
	550702	<5-10	

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[a] Excluding index cases, the total number of individuals susceptible to any virus was 82, the number infected after introduction of a virus into a household was 20 (25%).

c = coxsackievirus, E = echovirus